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THE
AMERICAN NATURALIST

VOL. LVII *November-December, 1923* No. 653

# TRISOMIC INHERITANCE IN THE POINSETTIA MUTANT OF DATURA

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## INTRODUCTION

THE phenomenon of mutation in the jimson-weed (*Datura Stramonium*) has already been discussed in earlier publications (1–10), in which it has been shown that the presence of an extra chromosome exerts an unbalancing effect upon the make-up of the individual plant. That the extra chromosome is a member of a particular chromosomal set and exerts its influence in $(2n+1)$ mutants by virtue of the specific factors which it contains was first clearly indicated by unexpected color ratios obtained in breeding the mutant Poinsettia. Earlier publications (6, 3, 4) have given an outline of the evidence then available which had led to the conclusion that the Poinsettia mutant, as well as its variety Wiry, has its extra chromosome in the set carrying the factors for purple and white flower color. The present paper will give the results of a further and more detailed study of the inheritance of color in this mutant which amply justify, we believe, the conclusion reached from the earlier and less extensive data. Inasmuch as a belief in the specificity of the extra chromosomes associated with the various unbalanced mutant types has been the starting point for a series of investigations in Datura, the basis for this belief seems to warrant a thorough investigation. The reason Poinsettia was chosen for intensive study rather than the mutant Cocklebur, in which the extra chromosome appar-

ently has been identified as a member of the set carrying the factors for armed and inermis capsules, is because plants can be classified for color characters at an early stage—in fact, as soon as the seedlings are above the ground—while plants must be grown to the fruiting stage before the type of capsule is discernible.

### DESCRIPTION OF POINSETTIAS

When grown in the field the adult Poinsettia is the most striking in appearance of all our mutants. Its long narrow dark-green leaves are clustered at the ends of branches in a fashion which was suggestive of the plant for which it has been named (5, Fig. 14). Towards the latter part of the growing season, masses of adventitious growth extrude in longitudinal lines from young stems, causing irregularities in the branching. Its internal anatomy (10) differs radically from the normal type. The capsule is nearly spherical, large and beset with long spines (4, Figs. 4 and 5). Frequently the floral parts are malformed by an increase in the number of stamens, lobes of calyx and corolla and divisions of the pistil. It is often difficult to induce the capsules to set on plants grown in the field, but less difficulty is experienced in obtaining seeds from plants grown in pots in the greenhouse.

FIG. 1. Two seedling Poinsettias in center in 3-inch pots with normals from same fraternity on either side, photographed at stage before plants are recorded.

Pot-grown Poinsettias (Fig. 1) are not so distinct as field-grown plants, but can readily be distinguished by

one familiar with their appearance. The leaves of seedlings are longer and narrower, have relatively fewer teeth and are slightly more depressed at the veins in comparison with normals. The majority of our Poinsettia cultures have been grown in the greenhouse and recorded from three-inch pots for reasons of economy. In all cases where doubt has arisen as to the classification of such seedlings, the plants in question have been grown to a stage when they could be recorded with certainty. We do not believe, therefore, that increased accuracy would have resulted from growing our Poinsettia cultures in the open where the numbers that could have been conveniently handled would have been greatly diminished.

### Inheritance of Poinsettia Complex

The inheritance of the Poinsettia complex resolves itself into the transmission, through the male and female gametes, of the extra chromosome which brings about the peculiarities of this $(2n+1)$ mutant. If the $(n+1)$ gametes functioned equally with the $n$ gametes and the $(2n+1)$ zygotes had the same survival value as the $2n$ zygotes, we should expect from selfing Poinsettias the following offspring: $1(2n+2\mathrm{P}n)+2(2n+1\mathrm{P}n)+1(2n)$, and from crossing Poinsettias with normals we should expect $1(2n+1)+1(2n)$. That the numbers of $2n$ and of $(2n+1)$ offspring from the latter combination are not equal in Table I is apparently due in Poinsettia as in Globe (2) to the unbalancing effect exercised by the extra chromosome upon the female gamete and the zygote. The decrease in vigor thus affected is perhaps somewhat less severe in Poinsettia than in Globe. The unbalancing effect is naturally greater in the gametes (1 vs $n$) than in the zygote (1 vs $2n$) and apparently causes an abortion of the pollen grains (3, Table I) or a retardation of the growth of the pollen tubes (9) affected. The result is that few if any pollen grains transmit the Poinsettia complex. This fact would tend to prevent the appearance of $(2n+2)$ Poinsettias which, judging from the $(2n+2)$ Globes, might be expected to have a low survival value.

TABLE I

INHERITANCE OF POINSETTIA COMPLEX WITHIN LINES 1 AND 2

| | Poinsettia Selfed | Poinsettia × Poinsettia | Poinsettia × Normal | Normal × Poinsettia |
|---|---|---|---|---|
| Number of Seed Parents | 75 | 32 | 41 | 2 |
| Normals | 5316 | 3041 | 2864 | 1126 |
| Poinsettias | 2098 | 1384 | 1304 | 0 |
| Per cent. Poinsettias | 28.30 ± .353 | 31.28 ± .470 | 31.29 ± .484 | 0.00 |

Percentage difference between offspring from Poinsettia selfed and from Poinsettia × Normal = 2.99 ± .599. Diff./P.E. Diff. = 4.99.

Difference between offspring from Poinsettia selfed and from Poinsettia × Poinsettia = 2.98 ± .588. Diff./P.E. Diff. = 5.07.

The extra chromosome is transmitted to slightly fewer offspring when a Poinsettia is selfed than when it is crossed with a normal or with another Poinsettia as pollen parent. The difference is not great but seems to be significant statistically. A similar but greater difference was found in Globe crosses (2) and may most reasonably be attributed to a difference in vigor of zygotes obtained respectively from selfing and from crossing. In the Globe crosses several diverse lines were involved, while in the Poinsettia crosses shown in the table only two closely similar lines (Lines 1 and 2) are represented.

## OCCURRENCE OF NEW POINSETTIAS

Poinsettia is not an uncommon form among our ($2n + 1$) mutants. In Table II are shown the data on its occurrence in line 1 derived from Washington, D. C.,

TABLE II

OCCURRENCE OF NEW POINSETTIAS FROM SELFING PLANTS IN LINE 1

| Type of Seed Parent | Number of Seed Parents | Normals | Poinsettias | Per cent. Poinsettias | Years Recorded |
|---|---|---|---|---|---|
| 2n | 16 | 1707 | 1 | 0.06 ± .040 | 1919–22 |
| 2n (Mass Planting) | 48 | 27616 | 14 | 0.05 ± .009 | 1919–21 |
| (2n + 1) | 54 | 4921 | 5 | 0.10 ± .030 | 1920–22 |
| (2n + 1) (Mass Planting) | 25 | 3242 | 0 | 0.00 | 1921 |

which has been used more than any other line in our breeding experiments. When seedlings are crowded in the field, the number of the various mutants reaching recordable age is considerably lessened. In consequence, mass-plantings are separated in the table from plantings in which the seedlings are potted up and handled individually. The number of plants so far recorded in line 1 is too small to warrant as yet any very definite conclusion in regard to the relative frequency of occurrence of the different mutants. Further data also will be necessary to decide whether or not there is any significance in the slightly increased proportion of Poinsettias and Globes (2) from $(2n+1)$ parents over that from normal $2n$ parents. It may be possible to present more significant figures at a later date when more evidence will have accumulated.

It may be mentioned that, in addition to the plants recorded in Table I which came from parents handled in the usual manner, we have secured Poinsettias and other mutants in increased numbers after subjecting parents to lowered temperatures. Due to the process of trisomic assortment, Poinsettias along with other $(2n+1)$ types also have appeared in the offspring from triploid parents (3, 4, 1).

There are apparently two factors controlling the number of new Poinsettia mutants: (1) the frequency of nondisjunction in the Poinsettia set of chromosomes; (2) the ability of the gamete or zygote to function under competition when burdened with the extra chromosome. Nondisjunction in the formation of the male gametes can have little or no influence upon the occurrence of $(2n+1\mathrm{P}n)$ plants since, as has been shown in the preceding section, the pollen is not effective to any considerable extent in transmitting the extra chromosome. In this respect the Poinsettia is possibly at a slight disadvantage in comparison with the Globe which is transmitted through its pollen to about 2 per cent. of its offspring. On the other hand, the survival value of egg cells or

zygotes with the extra Poinsettia chromosome seems, from data in Table I, to be slightly greater than that for the Globe. The differences in these respects between the two mutants, however, are at best slight and the data are not absolutely comparable.

### FORMULAE FOR TRISOMIC INHERITANCE

The ratios in ordinary Mendelian inheritance are disomic, being based upon the facts of assortment of the homologous chromosomes which have been associated together in disomes. An entirely different series of ratios will obviously result if the chromosomes bearing the factors in question are associated in trisomes or sets of three. Before considering the detailed breeding data, it will be well to present the theoretical ratios for trisomic inheritance. This is done in Table III.

In deriving the ratios, two assumptions have been necessary: (1) random assortment of the chromosomes in the trisome, (2) failure of the extra chromosome to be transmitted through the pollen. That transmission of the extra chromosome through the pollen can be neglected is obvious from the results in Table I. The detailed pedigrees given below show that both assumptions are justified for the material treated in the present paper. A $(2n + 1)$ plant with the allelomorphic pair Aa borne by the chromosomes in a trisome will, if duplex for A, have the formula AAa. On account of the large number of homologous chromosomes that may be associated in a single set—as many as seven in a $(4n + 3)$ Globe—it has been found convenient to use subscripts in our formulae and to write a duplex plant $A_2a$. The female gametes of such a plant will be $2A + a + A_2 + 2Aa$ and the male gametes, because $(n + 1)$ pollen does not function, will be $2A + a$. Thus selfing a duplex parent should give only A plants in the $(2n + 1)$ offspring and 8A to 1a plant in the $2n$ offspring. The formulae for other genetic types are given in the table and require no further discussion.[1]

[1] Through an uncorrected error in an earlier publication (6) the formula for the female gametes of a duplex Poinsettia was written $2P + p + pp + 2Pp$ instead of $2P + p + PP + 2Pp$.

TABLE III

TRISOMIC INHERITANCE OF AN ALLELOMORPHIC PAIR A,a IN A (2n + 1) MUTANT WHEN THE EXTRA CHROMOSOME IS NOT CARRIED BY THE POLLEN. FORMULAE FOR PARENTS, GAMETES AND BOTH 2n AND (2n + 1) OFFSPRING

| Female Parent (gametes in parenthesis) | Pollinated by | 2n offspring | | | | (2n + 1) offspring | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $A_2$ | Aa | $a_2$ | Ratio A:a | Ratio A:a | $A_2$ | $A_2a$ | $Aa_2$ | $a_2$ |
| $A_2$ ($A_2$ + A) | $A_2$ | 1 | ..... | ..... | 1:0 | 1:0 | 1 | ..... | ..... | ..... |
| | $A_2a$ | 2 | 1 | ..... | 1:0 | 1:0 | 2 | 1 | ..... | ..... |
| | $Aa_2$ | 1 | 2 | ..... | 1:0 | 1:0 | 1 | 2 | ..... | ..... |
| | $a_2$ | ..... | 1 | ..... | 1:0 | 1:0 | ..... | 1 | ..... | ..... |
| | $A_2$ | 1 | ..... | ..... | 1:0 | 1:0 | 1 | ..... | ..... | ..... |
| | Aa | 1 | 1 | ..... | 1:0 | 1:0 | 1 | 1 | ..... | ..... |
| | $a_2$ | ..... | 1 | ..... | 1:0 | 1:0 | ..... | 1 | ..... | ..... |
| $A_2a$ (2A + a + $A_2$ + 2Aa) | $A_2$ | 2 | 1 | ..... | 1:0 | 1:0 | 1 | 2 | ..... | ..... |
| | $A_2a$ | 4 | 4 | 1 | 8:1 | 9:0 | 2 | 5 | 2 | ..... |
| | $Aa_2$ | 2 | 5 | 2 | 7:2 | 9:0 | 1 | 4 | 4 | ..... |
| | $a_2$ | ..... | 2 | 1 | 2:1 | 3:0 | ..... | 1 | 2 | ..... |
| | $A_2$ | 2 | 1 | ..... | 1:0 | 1:0 | 1 | 2 | ..... | ..... |
| | Aa | 2 | 3 | 1 | 5:1 | 6:0 | 1 | 3 | 2 | ..... |
| | $a_2$ | ..... | 2 | 1 | 2:1 | 3:0 | ..... | 1 | 2 | ..... |
| $Aa_2$ (A + 2a + 2Aa + $a_2$) | $A_2$ | 1 | 2 | ..... | 1:0 | 1:0 | ..... | 2 | 1 | ..... |
| | $A_2a$ | 2 | 5 | 2 | 7:2 | 8:1 | ..... | 4 | 4 | 1 |
| | $Aa_2$ | 1 | 4 | 4 | 5:4 | 7:2 | ..... | 2 | 5 | 2 |
| | $a_2$ | ..... | 1 | 2 | 1:2 | 2:1 | ..... | ..... | 2 | 1 |
| | $A_2$ | 1 | 2 | ..... | 1:0 | 1:0 | ..... | 2 | 1 | ..... |
| | Aa | 1 | 3 | 2 | 4:2 | 5:1 | ..... | 2 | 3 | 1 |
| | $a_2$ | ..... | 1 | 2 | 1:2 | 2:1 | ..... | ..... | 2 | 1 |
| $a_2$ (a + $a_2$) | $A_2$ | ..... | 1 | ..... | 1:0 | 1:0 | ..... | ..... | 1 | ..... |
| | $A_2a$ | ..... | 2 | 1 | 2:1 | 2:1 | ..... | ..... | 2 | 1 |
| | $Aa_2$ | ..... | 1 | 2 | 1:2 | 1:2 | ..... | ..... | 1 | 2 |
| | $a_2$ | ..... | ..... | 1 | 0:1 | 0:1 | ..... | ..... | ..... | 1 |
| | $A_2$ | ..... | 1 | ..... | 1:0 | 1:0 | ..... | ..... | 1 | ..... |
| | Aa | ..... | 1 | 1 | 1:1 | 1:1 | ..... | ..... | 1 | 1 |
| | $a_2$ | ..... | ..... | 1 | 0:1 | 0:1 | ..... | ..... | ..... | 1 |
| $A_2$ (A) | $A_2$ | 1 | ..... | ..... | 1:0 | | ..... | ..... | ..... | ..... |
| | $A_2a$ | 2 | 1 | ..... | 1:0 | | ..... | ..... | ..... | ..... |
| | $Aa_2$ | 1 | 2 | ..... | 1:0 | | ..... | ..... | ..... | ..... |
| | $a_2$ | ..... | 1 | ..... | 1:0 | | ..... | ..... | ..... | ..... |
| Aa (A + a) | $A_2$ | 1 | 1 | ..... | 1:0 | | ..... | ..... | ..... | ..... |
| | $A_2a$ | 2 | 3 | 1 | 5:1 | | ..... | ..... | ..... | ..... |
| | $Aa_2$ | 1 | 3 | 2 | 4:2 | | ..... | ..... | ..... | ..... |
| | $a_2$ | ..... | 1 | 1 | 1:1 | | ..... | ..... | ..... | ..... |
| $a_2$ (a) | $A_2$ | ..... | 1 | ..... | 1:0 | | ..... | ..... | ..... | ..... |
| | $A_2a$ | ..... | 2 | 1 | 2:1 | | ..... | ..... | ..... | ..... |
| | $Aa_2$ | ..... | 1 | 2 | 1:2 | | ..... | ..... | ..... | ..... |
| | $a_2$ | — | — | 1 | 0:1 | | ..... | ..... | ..... | ..... |

The table gives the phenotypic ratios as well as the genetic types of the zygotes resulting from the various

kinds of matings. It should be remembered that ($2n + 1$) individuals are always fewer than $2n$ individuals in the offspring and, therefore, the ratios can be expected to hold only within each type unless a correction is applied for differential viability.

### COLOR RATIOS FROM POINSETTIA MATINGS

The inheritance of flower color in Poinsettias can be used as a test of the assumptions upon which the theoretical ratios in Table III have been derived. The breeding data in the present paper will be confined to two closely related main lines from Washington, D. C., Line 1 being purple and Line 2 being white-flowered. At a later date it may be possible to consider certain consistent and predictable deviations from the calculated ratios when the factors for white are derived from another source than Line 2 (4). It seems best, however, to establish the rule firmly before considering the exceptions.

Table IV gives the detailed cultures from various types of Poinsettia matings which showed segregation. While considerable deviation from the calculated ratios of Table III occurs in the individual cultures, such deviation seems to be largely random. In the summarized data in Table V, the numbers are large enough to warrant the calculation of probable errors. It will be observed that in only part of one complete ratio is the deviation from the calculated value as large as three times the probable error —and in this case the numbers are relatively small. Furthermore the various types of matings show an excess of purples about as frequently as an excess of whites.

There is only a single exception which at first might seem serious. This is the occurrence of a white Poinsettia in a cross between a duplex ($P_2p$) female and a white ($p_3$) Poinsettia. There is no doubt about the classification of this plant as a white Poinsettia. Its appearance may be tentatively explained by the exceptional functioning of a $p_2$ pollen grain.

## Distribution of Genotypes

The figures in Tables IV and V show the distribution of purples and whites, but give no direct evidence in regard to the heterozygous purples. Tests on plants in the greenhouse seem to indicate that there is a decrease in the amount of purple pigment in the flowers from $P_3$ to $Pp_2$ individuals. Since, however, the intensity of floral pigment varies from day to day in the same genetic type, probably on account of the differences in illumination and other environmental factors, it has not been found

TABLE IV

Detailed Cultures from Various Types of Poinsettia (Pn.) Matings showing Segregation for Purple (P) and White (p) Flower Color

IV (a) Analysis of duplex Poinsettia ($P_2p$). [*Cf.* also (b)].

| $P_2p \times P_2p$ | | | | $P_2p \times p_2$ | | | | $P_2p \times p_3$ | | | | $P_2p \times Pp_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nor. | | Pn. | | Nor. | | Pn. | | Nor. | | Pn. | | Nor. | | Pn. | |
| P | p | P | p | P | p | P | p | P | p | P | p | P | p | P | p |
| 44 | 2 | 11 | | 27 | 11 | 22 | | 8 | 2 | 8 | | 21 | 3 | 4 | |
| 52 | 3 | 19 | | 40 | 23 | 30 | | 50 | 37 | 28 | | 74 | 28 | 39 | |
| 54 | 9 | 26 | | 53 | 25 | 48 | | 12 | 7 | 13 | 1 | 40 | 7 | 13 | |
| 71 | 7 | 42 | | 63 | 27 | 31 | | | | | | 77 | 26 | 23 | |
| 37 | 4 | 36 | | 31 | 17 | 18 | | | | | | 12 | 3 | 5 | |
| 76 | 10 | 21 | | 11 | 8 | 21 | | | | | | 32 | 18 | 21 | |
| 37 | 4 | 11 | | 48 | 14 | 33 | | | | | | 36 | 10 | 30 | |
| 20 | 5 | 20 | | 42 | 17 | 30 | | | | | | | | | |
| 85 | 6 | 11 | | 113 | 41 | 49 | | $p_3 \times P_2p$ | | | | $P_2p \times Pp$ | | | |
| 84 | 7 | 31 | | 39 | 18 | 46 | | Nor. | | Pn. | | Nor. | | Pn. | |
| 67 | 9 | 38 | | 27 | 10 | 18 | | P | p | P | p | P | p | P | p |
| 62 | 9 | 30 | | 27 | 11 | 17 | | 53 | 28 | 18 | 3 | 24 | 2 | 15 | |
| 32 | 4 | 17 | | 58 | 22 | 20 | | 22 | 9 | 7 | 11 | 35 | 4 | 37 | |
| 47 | 10 | 29 | | 66 | 37 | 26 | | 57 | 25 | 27 | 24 | 46 | 4 | 24 | |
| 14 | 3 | 0 | | | | | | 67 | 45 | 33 | 18 | 113 | 26 | 45 | |
| 131 | 21 | 74 | | | | | | 213 | 126 | 100 | 53 | | | | |
| 130 | 11 | 43 | | | | | | | | | | | | | |
| 86 | 14 | 9 | | $p_2 \times P_2p$ | | | | | | | | | | | |
| 62 | 5 | 9 | | Nor. | | Pn. | | | | | | | | | |
| 48 | 7 | 16 | | P | p | P | p | | | | | | | | |
| 58 | 7 | 20 | | | | | | | | | | | | | |
| 18 | 2 | 7 | | 71 | 47 | † | † | | | | | | | | |
| 92 | 10 | 41 | | 57 | 24 | | | | | | | | | | |
| 32 | 6 | 10 | | 74 | 49 | | | | | | | | | | |
| 113 | 10 | 14 | | 49 | 28 | | | | | | | | | | |
| 67 | 8 | 26 | | 299 | 164 | | | | | | | | | | |
| 45 | 10 | 23 | | 286 | 81 | | | | | | | | | | |
| 59 | 3 | 11 | | 406 | 235 | | | | | | | | | | |
| 39 | 6 | 24 | | 62 | 29 | | | | | | | | | | |
| 64 | 8 | 23 | | 660 | 383 | | | | | | | | | | |

IV (b) Analysis of simplex Poinsettia ($Pp_2$). [*Cf.* also (a)].

TABLE IV (*Continued*)

| $Pp_2 \times Pp_2$ | | | | $Pp_2 \times p_2$ | | | | $Pp_2 \times p_3$ | | | | $Pp_2 \times P_2p$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nor. | | Pn. | | Nor. | | Pn. | | Nor. | | Pn. | | Nor. | | Pn. | |
| P | p | P | p | P | p | P | p | P | p | P | p | P | p | P | p |
| 45 | 37 | 22 | 6 | 21 | 42 | 16 | 3 | 62 | 91 | 44 | 25 | 43 | 9 | 34 | 1 |
| 24 | 12 | 17 | 1 | 23 | 35 | 13 | 5 | 75 | 143 | 69 | 33 | 21 | 10 | 16 | 1 |
| 34 | 22 | 33 | 8 | 19 | 38 | 26 | 9 | 6 | 20 | 6 | 8 | 100 | 23 | 26 | 6 |
| 45 | 35 | 31 | 7 | 5 | 10 | 11 | 7 | 29 | 38 | 23 | 9 | 15 | 5 | 24 | 2 |
| 59 | 48 | 31 | 9 | 30 | 67 | 28 | 10 | | | | | 65 | 17 | 20 | 3 |
| 33 | 33 | 26 | 4 | 23 | 59 | 15 | 7 | | | | | 48 | 15 | 36 | 6 |
| 25 | 17 | 15 | 7 | 25 | 45 | 17 | 5 | | | | | 17 | 4 | 11 | 1 |
| 28 | 35 | 35 | 12 | 14 | 17 | 21 | 4 | | | | | 23 | 10 | 13 | 0 |
| 38 | 30 | 22 | 5 | 23 | 45 | 19 | 12 | | | | | 18 | 4 | 15 | 1 |
| 32 | 25 | 27 | 9 | 36 | 78 | 40 | 22 | | | | | 25 | 6 | 6 | 2 |
| 37 | 21 | 21 | 3 | | | | | | | | | 49 | 19 | 49 | 3 |
| 23 | 22 | 21 | 6 | | | | | | | | | 79 | 17 | 42 | 5 |
| 51 | 24 | 26 | 8 | | | | | | | | | 71 | 21 | 45 | 8 |
| 4 | 5 | 10 | 4 | | | | | | | | | | | | |
| 41 | 28 | 31 | 8 | | | | | | | | | | | | |
| 41 | 32 | 13 | 5 | | | | | | | | | | | | |
| 34 | 28 | 28 | 5 | | | | | | | | | | | | |
| 40 | 27 | 32 | 8 | | | | | | | | | | | | |
| 33 | 31 | 29 | 5 | | | | | | | | | | | | |
| 31 | 26 | 29 | 6 | | | | | | | | | | | | |
| 32 | 33 | 29 | 3 | | | | | | | | | | | | |
| 37 | 27 | 27 | 10 | | | | | | | | | | | | |
| 29 | 25 | 33 | 8 | | | | | | | | | | | | |
| 24 | 19 | 19 | 6 | | | | | | | | | | | | |
| 66 | 52 | 33 | 12 | | | | | | | | | | | | |
| 77 | 40 | 10 | 2 | | | | | | | | | | | | |
| 64 | 53 | 29 | 8 | | | | | | | | | | | | |
| 48 | 43 | 23 | 7 | | | | | | | | | | | | |
| 47 | 30 | 27 | 4 | | | | | | | | | | | | |
| 34 | 30 | 37 | 15 | | | | | | | | | | | | |
| 21 | 19 | 16 | 2 | | | | | | | | | | | | |
| 31 | 24 | 17 | 5 | | | | | | | | | | | | |
| 1 | 0 | 2 | 2 | | | | | | | | | | | | |
| 21 | 18 | 20 | 8 | | | | | | | | | | | | |
| 40 | 24 | 29 | 2 | | | | | | | | | | | | |
| 17 | 20 | 20 | 3 | | | | | | | | | | | | |

| $p_3 \times Pp_2$ | | | | $Pp_2 \times Pp$ | | | |
|---|---|---|---|---|---|---|---|
| Nor. | | Pn. | | Nor. | | Pn. | |
| P | p | P | p | P | p | P | p |
| 77 | 121 | 28 | 46 | 17 | 8 | 17 | 7 |
| 41 | 106 | 19 | 49 | 24 | 12 | 10 | 0 |
| 19 | 45 | 10 | 24 | 45 | 19 | 21 | 3 |
| 9 | 34 | 4 | 10 | 52 | 24 | 19 | 4 |
| 45 | 98 | 15 | 55 | 63 | 39 | 44 | 10 |
| 12 | 14 | 5 | 7 | 108 | 61 | 74 | 10 |
| 19 | 18 | 7 | 10 | 47 | 33 | 33 | 6 |

| $Pp_2 \times p_2$ | | | |
|---|---|---|---|
| Nor. | | Pn. | |
| P | p | P | p |
| 4 | 9 | † | † |
| 59 | 102 | | |
| 51 | 99 | | |
| 82 | 181 | | |
| 49 | 127 | | |
| 60 | 131 | | |
| 43 | 84 | | |
| 10 | 22 | | |
| 52 | 112 | | |
| 28 | 51 | | |
| 37 | 77 | | |
| 45 | 89 | | |
| 61 | 137 | | |
| 147 | 270 | | |
| 122 | 286 | | |
| 91 | 190 | | |
| 225 | 425 | | |

IV (c) Analysis of nulliplex Poinsettia ($p_3$). [*Cf.* also (a) and (b)].

| $p_3 \times Pp$ | | | |
|---|---|---|---|
| Nor. | | Pn. | |
| P | p | P | p |
| 24 | 22 | 22 | 19 |
| 42 | 38 | 7 | 11 |
| 22 | 35 | 9 | 8 |
| 11 | 8 | 5 | 8 |
| 26 | 29 | 12 | 21 |
| 45 | 43 | 11 | 28 |

(† Seedlings from the cross Normal × Poinsettia were not grown to a stage in which Poinsettia plants could be identified since, as shown in Table I, the extra Poinsettia chromosome appears not to be transmitted to any considerable extent through the pollen.)

possible to classify the different genetic types of purple by inspection with any accuracy. If the disturbing factors could be rendered constant, we believe the presence of different numbers of genes for purple would be reflected in the flower color. With the conditions under which the plants are grown, however, it is necessary to obtain color records of their progeny in order to classify with certainty the genetic constitution of purples.

The breeding test of normals is very simple. Since mutants are not involved, the individuals to be tested can be selfed and the purple and white seedlings recorded and discarded as soon as they appear in the seed pans. Purple-flowered plants have red-stemmed and white-flowered plants have green-stemmed seedlings. Large numbers are not necessary, since purple normals are either $P_2$ or Pp, and the ratios to be distinguished are all

TABLE V

SUMMARY OF COLOR RECORDS FROM POINSETTIA PARENTS. P = PURPLE. p = WHITE

(For details of segregating cultures *cf.* Table IV.)

| Type of Pollination | Number of Seed Parents | Normals | | | Poinsettias | | |
|---|---|---|---|---|---|---|---|
| | | P | p | Dev./P.E | P | p | Dev./P.E. |
| $P_2 \times P_2$ | 10 | 882 | 0 | | 263 | 0 | |
| *Calculated* | | *882* | *0* | ..... | *263* | *0* | ..... |
| $P_2p \times P_2p$ | 29 | 1826 | 220 | | 692 | 0 | |
| *Calculated* | | *1818.7* | *227.3 ± 9.58* | *0.8* | *692* | *0* | ..... |
| $P_2p \times P_2$ | 14 | 645 | 281 | | 409 | 0 | |
| *Calculated* | | *617.3* | *308.7 ± 9.66* | *2.9* | *409* | *0* | ..... |
| $P_2p \times P_2$ | 2 | 70 | 46 | | 49 | 1 | |
| *Calculated* | | *77.3* | *38.7 ± 3.42* | *2.1* | *50* | *0* | ..... |
| $P_2p \times Pp_2$ | 7 | 292 | 95 | | 135 | 0 | |
| *Calculated* | | *301* | *86 ± 5.51* | *1.6* | *135* | *0* | ..... |
| $P_2p \times Pp$ | 4 | 218 | 36 | | 121 | 0 | |
| *Calculated* | | *211.8* | *42.2 ± 4.01* | *1.5* | *121* | *0* | ..... |
| $P_2 \times P_2p$ | 9 | 1958 | 1040 | | † | † | |
| *Calculated* | | *1998.7* | *999.3 ± 17.39* | *2.3* | | | |
| $P_2 \times P_2p$ | 2 | 412 | 233 | | 185 | 109 | |
| *Calculated* | | *430* | *215 ± 8.07* | *2.2* | *196* | *98 ± 5.45* | *2.0* |
| $Pp_2 \times Pp_2$ | 35 | 1287 | 995 | | 870 | 223 | |
| *Calculated* | | *1267.8* | *1014.2 ± 16.01* | *1.2* | *850.1* | *242.9 ± 9.27* | *2.1* |
| $Pp_2 \times P_2$ | 10 | 219 | 436 | | 206 | 84 | |
| *Calculated* | | *218.3* | *436.7 ± 8.13* | *0.1* | *193.3* | *96.7 ± 5.41* | *2.3* |
| $Pp_2 \times P_2$ | 4 | 172 | 292 | | 142 | 75 | |
| *Calculated* | | *154.7* | *309.3 ± 6.84* | *2.5* | *144.7* | *72.3 ± 4.68* | *0.6* |
| $Pp_2 \times P_2p$ | 13 | 574 | 160 | | 337 | 39 | |
| *Calculated* | | *570.9* | *163.1 ± 7.59* | *0.4* | *334.2* | *41.8 ± 4.11* | *0.7* |
| $Pp_2 \times Pp$ | 7 | 356 | 196 | | 218 | 40 | |
| *Calculated* | | *368* | *184 ± 7.46* | *1.6* | *215* | *43 ± 4.04* | *0.7* |
| $P_2 \times Pp_2$ | 12 | 1166 | 2392 | | † | † | |
| *Calculated* | | *1186* | *2372 ± 18.94* | *1.1* | | | ..... |
| $P_2 \times Pp_2$ | 3 | 222 | 436 | | 88 | 201 | |
| *Calculated* | | *219.3* | *438.7 ± 8.15* | *0.3* | *96.3* | *192.7 ± 5.40* | *1.5* |
| $P_2 \times P_2$ | 1 | 0 | 143 | | 0 | 73 | |
| *Calculated* | | *0* | *143* | ..... | *0* | *73* | ..... |
| $P_2 \times Pp$ | | 170 | 175 | | 66 | 95 | |
| *Calculated* | 6 | *172.5* | *172.5 ± 6.27* | *0.4* | *80.5* | *80.5 ± 4.28* | *3.4* |

(† See footnote Table IV.)

to none and 3:1. The average number of seedlings in cultures from the $P_2$ offspring of Table VI was 84.5. The smallest number was 23. Since there is only 1 chance in 1000 (8) of this smallest culture actually representing a 3:1 ratio, our classification of genotypes among normals may be considered accurate.

TABLE VI

DISTRIBUTION OF GENOTYPES AMONG OFFSPRING FROM SELFED POINSETTIAS

| Parent | Offspring | | | | |
|---|---|---|---|---|---|
| | Normals | | Poinsettias | | |
| | $P_2$ | Pp | $P_2$ | $P_2p$ | $Pp_2$ |
| $P_2$ | 23 | 0 | 10 | 0 | 0 |
| *Calculated* | *23* | *0* | *10* | *0* | *0* |
| $P_2p$ | 57 | 54 | 12 | 30 | 13 |
| *Calculated* | *55.5* | *55.5* | *12.2* | *30.6* | *12.2* |
| $Pp_2$ | 24 | 91 | 0 | 14 | 24 |
| *Calculated* | *23* | *92* | *0* | *10.9* | *27.1* |

The breeding test of Poinsettias is somewhat more tedious than that of normals if the seedlings are grown to an age when Poinsettias can be distinguished. It is not necessary to distinguish the Poinsettias, however, as a consideration of the expected ratios will show. In the first place, $P_2$ individuals which should throw all purples are to be distinguished from duplex ($P_2p$) or simplex ($Pp_2$) heterozygous individuals. The duplex heterozygous individuals ($P_2p$) throw the more purples and alone of the two ratios need concern us here. The ratio expected is 8P:1p among the normals and 9P:0p among the Poinsettias. This complete ratio assumes that as many Poinsettias as normal zygotes come to recordable age. Such an assumption has never been realized in our experience when any considerable numbers were involved. If we make the assumption of this extreme condition of an equality of normals and Poinsettias in the offspring which would give the maximum number of purples from a $P_2p$ individual, our problem then is to differ-

entiate between an all to none and an 8:1 ratio in a population represented by half the number of seedlings in the given culture. The average number of seedlings from the $P_3$ Poinsettia offspring of Table VI was 177.8. The smallest number was 105. Assuming one half, or 52, to have been normals, there are only 2 chances in 1000 (8) that the culture under discussion represents an 8:1 ratio and that the plant from which it was derived was not homozygous ($P_3$). When it is remembered that the conventional "3 times the probable error" represents 43 chances in 1000, it is seen that our smallest culture from a $P_3$ individual stands little chance of having been wrongly classified.

The distinction between a $P_2p$ and a $Pp_2$ individual can also readily be made without recording the offspring for Poinsettias. The ratio from a $P_2p$ individual varies between 17P:1p, the extreme limit on one side assuming that the Poinsettias are equal to the normals, and 8P:1p, the extreme limit on the other side assuming that no Poinsettias survive. In like manner it may be shown that the ratios from a $Pp_2$ individual lie between the limits 2P:1p and 5P:4p. Our most difficult task possible in consequence would be to differentiate between an 8:1 and a 2:1 ratio. The classification, therefore, of the three types of purple Poinsettias by recording and discarding their young offspring from the seed pans runs counter to no mathematical principles and has given no difficulties in actual practice.

For the sake of their inclusion in Table IV all the Poinsettia offspring from $Pp_2$ parents in Table VI had their progeny classified for both Poinsettias and normals as well as the Poinsettia offspring of 18 of the $P_2p$ parents. Of the latter, 5 were $P_3$, 8 $P_2p$ and 5 Pp.

The distribution of genotypes shown in Table VI is in perfect accord statistically with the visible ratios shown in Tables IV and V. Together they form a compelling mass of evidence, we believe, that the extra chromosome in the Poinsettia mutant of Datura is a member of the set carrying the genes for purple and white flower color.

When more Mendelian characters are available it may be possible to label the chromosomes in the other $(2n+1)$ mutants by the factors which they contain. The data in the present paper, although dealing with the extra chromosome in but a single mutant, offer presumptive evidence for the specificity of the extra chromosome in all the other $(2n+1)$ mutants.

## SUMMARY

(1) The $(2n+1)$ mutant Poinsettia in *Datura Stramonium* is briefly described.

(2) The frequency of its new occurrence in offspring of $2n$ parents is about once in two to three thousand individuals.

(3) The extra chromosome responsible for the peculiarities of the Poinsettia mutant, (a) is not transmitted through the pollen or to only a slight extent; (b) is transmitted through only about 30 per cent. of the egg cells; (c) is transmitted to a slightly larger proportion of the offspring when the Poinsettia parent is pollinated from a normal or from another Poinsettia than when it is selfed.

(4) It is, therefore, concluded: (a) that the condition in the Poinsettia is similar to that previously found for the Globe mutant in that gametes with the extra chromosome are less viable than those with the normal $(n)$ number, and (b) that crossing the mutant with a normal or with another Poinsettia in comparison with selfing probably gives increased vigor and therefore increased survival value to the $(2n+1)$ zygotes which result.

(5) The theoretical ratios are given for trisomic inheritance based upon non-transmission of the extra chromosome through the pollen and upon the assumption of random assortment of chromosomes within the trisome.

(6) Both the phenotypic and genetic ratios for the inheritance of purple pigmentation in the offspring of Poinsettia parents agree closely with the theoretical ratios.

(7) It is therefore concluded: (a) that Poinsettia has its extra chromosome in the set which carries the genes for purple and white flower color, and (b) that the three chromosomes in this set assort at random.

LITERATURE CITED

1. Belling, John, and A. F. Blakeslee. 1922. The Assortment of Chromosomes in Triploid Daturas. AMER. NAT., 56: 339–346.
2. Blakeslee, A. F. 1921. The Globe Mutant in the Jimson Weed. *Genetics,* 6:241–264.
3. Blakeslee, A. F. 1921. Types of Mutations and Their Possible Significance in Evolution. AMER. NAT., 55:254–267.
4. Blakeslee, A. F. 1922. Variations in Datura Due to Changes in Chromosome Number. AMER. NAT., 56:16–31.
5. Blakeslee, A. F., and B. T. Avery, Jr. 1919. Mutations in the Jimson Weed. *Jour. Heredity,* 10:111–120.
6. Blakeslee, A. F., John Belling and M. E. Farnham. 1920. Chromosomal Duplication and Mendelian Phenomena in Datura Mutants. *Science,* N.S., 52:388–390.
7. Blakeslee, A. F., John Belling, M. E. Farnham and A. D. Bergner. 1922. A Haploid Mutant in the Jimson Weed, Datura Stramonium. *Science,* N.S., 55:646–647.
8. Blakeslee, A. F., John Belling and J. Arthur Harris. 1922. The Probability Established by a Culture of Given Size That a Mating is Capable of Producing Only Dominant Individuals. AMER. NAT., 56: 458–461.
9. Buchholz, John T. and A. F. Blakeslee. 1922. Studies of the Pollen Tubes and Abortive Ovules of the Globe Mutant of Datura. *Science,* N.S., 55:597–599.
10. Sinnott, Edmund W. and A. F. Blakeslee. 1922. Structural Changes Associated with Factor Mutations and with Chromosome Mutations in Datura. *Proc.* Nat. Acad. Sc., 8:17–19.

# THE ORIGIN OF CYCLOPEAN MONSTERS

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## INTRODUCTION

IN times past the occasional birth of monsters gave rise to strange myths of races of peoples of peculiar structure. It is quite possible that the one-legged folk described by Pliny were imagined as a result of seeing a case of monopodia or perhaps sympodia. Again, it is possible that the ancient Cyclops or Polyphemus with his one-eyed subjects was the reaction of the ancients to the cyclopean foetus.

Cyclocephalus, synapsia or cyclopia was first described by Lycosthenes in his Chronicum (1557). The specimen figured had a single eye above a normal nose. Licetus (De Monstris, p. 133, 1668) reported a monster with a mesial eye with two pupils and with a nasal proboscis above the eye. He also mentioned a Janus double monster with the same cyclopean deformity. Since the early part of the eighteenth century, many cases of cyclopia in mammals have been recorded, but in few of the cases have the descriptions been complete and the clinical data included.

Classifications of cyclopean monsters have been made by Geoffroy St. Hilaire, Ahlfeld and more recently by H. H. Wilder ('08). Stockard has shown experimentally that in the cyclopean fish we may have a regular gradation from a single median eye to an hour glass eye, and finally to two eyes close together, then to the normal two eyes. Stockard ('09) has distinguished cyclopia from the monophthalmic condition sometimes present. In this condition the single eye is in a lateral position.

## OCCURRENCE AND VIABILITY OF CYCLOPEAN MONSTERS

Among the mammals, cyclopia has been reported in man, monkey, cow, sheep, goat, pig, stag, horse, dog, cat,

rabbit and rat. Cyclopia has also been recorded in birds —chick, goose, dove, duck—and in fish—salmon, skate, trout. Cyclopean monsters have been experimentally produced in the chick, frog, salamander, fish and the flatworm, Planaria.

In many of the cyclopean monsters there are no abnormalities other than those of the head. In some the mouth is normal. In man, the oldest cyclops recorded by Valenti ('94) lived seventy-three hours. In the skate described by Paolucci ('74) the age was several years, since the fish was 1½ feet long and 2½ feet wide. The fish seemed able to procure its food readily.

Yung ('01) reports a case of monophthalmia in a rainbow trout. The eye was on the left and apparently normal. The fish lived for 22 months, feeding on Tubifex. Movement was in circles. Stockard ('10) succeeded in keeping Magnesium embryos for one month in aquaria. He did not attempt to keep them longer. In cases of cyclopia in which the mouth is normal there should not be great difficulty in keeping them alive. Many of the human cyclops breathe through the mouth for a few minutes.

## General External Appearance of the Mammalian Cyclops

In cyclopia there is generally a defect of the nose, and the eye is found in the median line of the face. Sometimes the mouth is defective or even fused with the ears.

It might be profitable to consider the classification of specimens of cyclopia made by Saint Hilaire and Ahlfeld.

Geoffroy Saint Hilaire classified specimens of cyclocephalus or cyclopia as follows:

(1) Ethmocephalus.
Olfactory organs not completely atrophied. Nose represented as proboscis with two imperfect nostrils, or a single opening. There are two eyes and two eyesockets.

(2) Cebocephalus.
No nose nor representation of it. Interocular region is narrow and perfectly flat, but two eyes and two orbital cavities are present.

(3) Rhinocephalus.

In rhinocephalus the nose is represented by a proboscis inserted at the base of the forehead. Beneath this proboscis is a single orbital cavity containing two orbits more or less completely fused.

(4) Cyclocephalus.

In cyclopia proper there is a fusion of the orbital cavities and of the eyes without a vestige of a nose or of a proboscis.

(5) Stomocephalus.

In stomocephalus there is the same deformity as in rhinocephalus or in cyclocephalus; but, unlike these the lower portion of the face is affected. The upper and lower maxillary bones are very defective but the normal amount of skin is provided, so that the latter, being in excess of the needs of the lower face, hangs in folds, especially at the region of the mouth, resembling somewhat the proboscis of the rhinocephalus.

The classification of Ahlfeld,[1] which most persons now accept, is based on the fact that the specimens may be placed in a series ranging from single orbital cavity, with no eye at all, to two perfect eyes close together.

Ahlfeld's classification of cyclopean monsters:

(1) The eyes approach one another, the face is narrow, the nose is rudimentary.

(2) There is a close approach of the eyes to one another. There is a rudiment of nose, but it is still between the eyes.

(3) There is a union of the orbital cavities at their inner edge, but the eyelids still extend across the face on both sides. The nose is represented by a cylindrical body above the junction of the eye-sockets composed of the skin structure of the nose, with possibly some cartilaginous formation at the base, or pedicle.

(4) There is a complete union of the orbital cavities and a junction of the two orbits, the single eyeball, however, possessing two corneae, two pupils and two lenses. There may be an entire absence of the proboscis, but it is usually present, always above the single eye.

(5) The corneae of the single eyeball are united into a figure-of-eight space. The fused eyelids form a rhomboidal figure. There may still be two pupils and two lenses, the latter may be single but larger than common, and elongated in its transverse diameter.

(6) The cornea is not formed at all. The sclera covers the whole eyeball, and fills out the space between the two eyelids.

(7) The eyesocket is very ill developed and the orbit rudimentary or altogether absent. (Anophthalmia cyclopica.)

## THE EYE, OPTIC NERVE AND EYE-MUSCLES

Under cyclopia have been classed all gradations from

[1] These classifications are to be found in Hirst and Piersol's "Human Monstrosities." Philadelphia, 1892.

two eyeballs side by side in a single orbit to an hour-glass eye, then down to a single eye, with apparently no traces of duplication, and finally to the complete absence of the eye. There may be no lens or cornea or there may be a deeply buried lens. Generally there are four eyelids. Sometimes there are two nerves unfused, sometimes the single optic nerve is formed by the fusion of the fibers just before the two eyes are reached.

*The eye-muscles:* Owing to their position, the internal recti are generally absent. The obliques may be present. The external recti are generally present.

### The Nasal Proboscis

In many cases of cyclopia there is a nasal proboscis, generally situated above the median eye. The proboscis is generally fleshy, and may be extremely short, or as long as the ordinary nose. At its extremity is seen a fleshy opening (sometimes two) which leads into a canal containing sebaceous and mucous material. In some probosces there has been found a rod of cartilage. No olfactory nerve is found in the proboscis.

Sometimes two probosces are found. Taruffi and Vogt ('94) described a foetus in which there were two probosces one above the eye and another below it. Devaine and Robin (Ballantyne, '04) and Wilder ('08) have described similar cases in pigs in which there were both proboscis and nose. Otto described a case of sub-orbital proboscis in a lamb. Allan ('48) has described the only case that the writer knows of a human foetus with the proboscis below the eye. This specimen is a case of cyclopean otocephalus with sub-orbital proboscis.

There is a remarkable similarity between the cyclops with a proboscis and the elephant. In both cases the growth is similar. In the elephant the basal elements of the trunk and of the proboscis are separate, but subsequently unite.

### Deformities of the Skull accompanying Cyclopia

In man, hydrocephalus is a more frequent adjunct to cy-

clopia than in lower animals. When hydrocephalus accompanies cyclopia the anterior fontanelles are widely separated and the bones of the skull are thin. In cases of cyclopia unaccompanied by hydrocephalus, the bones are thick and the fontanelles are closed. (Wyman, '59.) The posterior halves of the parietals are separated, the opening being closed by a thick membrane. Generally the following bones are absent: Ethmoid, presphenoid, nasal process of the superior maxillary, lachrymals, vomer and inferior turbinated. The frontal bones are usually unfused.

The nose is represented by a fleshy teat-like process, which is generally situated above the eye. In cases in which the proboscis is absent, the ears are frequently fused (otocephalus) and the mouth is not well defined. The palate may or may not be cleft.

In the pig, according to Wyman ('59), the cranium is separated from the bones of the face and thrown upward so that the roof of the orbit is nearly vertical and extends transversely across the face. The frontal, ethmoid, vomer and nasal bones are generally absent. The superior maxillary is short and frequently recurved. Wedekind and others speak of the absence of the premaxillaries, but Wyman thinks that these are probably not absent, but displaced and represented by a pair of bones found in the snout. Wyman cites the embryological fact that the premaxillaries are developed independently from the median bud, while the maxillaries project from the first visceral arch and subsequently unite with the premaxillaries. Wyman's theory is that a cyclopean eye, projecting forward in the median line, would have a tendency to throw the premaxillary bud upward and thus prevent its union with the maxillaries. In the absence of the ethmoid, the orbital plates of the frontal meet on the median line.

In otocephalus the ears approach one another and in some specimens are united (Chidester, '14). The lower jaw is rudimentary or sometimes absent entirely. The mouth is poorly developed and may be fused with the

openings of the ears. Otocephalus is extremely rare in man. One of the cases of otocephalus in the human foetus was described by Smith and Parker ('82). In this case the mouth was but 3 mm in diameter. The tongue may be rudimentary or absent. The hyoid bone may be perfect or completely absent.

The cause of otocephalus is supposed to be an arrest of the development of the lower processes (Meckel's cartilages) of the first visceral arch. Dareste held ('77, '91) that the primary cause was an arrest in the development of the cephalic fold of the amnion.

### The Brain and the Cranial Nerves

In fish, Stockard ('10) noted that sometimes the brain of a cyclopean monster is only very slightly malformed. In the mammalian cyclops, however, we generally find an extremely malformed brain. In many cases there is hydrocephalus and almost invariably the cerebrum is defective. It is sometimes incompletely divided into two hemispheres, sometimes there are three vesicles (Chidester, '14), and very frequently there is no division into hemispheres. The parts which develop from the first cerebral vesicle are extremely defective. The falx cerebri is defective or absent. The corpora quadrigemina, remarkably enough, are ordinarily almost normal. The corpora striata are generally imperfect. The crus cerebri is generally single. The fornix, septum lucidum and corpus callosum are generally absent, though Banchi ('05) reported a case in which they were normal and the corpora striata were present in good condition. The third and lateral ventricles are commonly fused together. This was the case in Banchi's specimens, though both fornix and corpus callosum were present. The epiphysis is frequently absent, but the hypophysis is almost always present.

There is great variation in the occurrence of an optic nerve. In the case of the absence of the eyeball, the optic nerve is generally absent. With fused eyes, the optic nerves may be separate; or there may be a fusion until

just before the eyeballs are entered; or there may be but one nerve. Sometimes well-developed eyeballs are not accompanied by the formation of a nerve. The olfactory nerves are frequently absent in almost any stage of cyclopia. The third, fourth and sixth nerves are frequently small, but generally present. The other cranial nerves are generally present.

The Circle of Willis has been figured in but few cases of cyclopia on record. Cleland ('77) reports two cases of cyclopia in the pig in which a mesial artery instead of a pair of internal carotids pierced the dura mater and joined the basilar. Phisalix ('89) gives drawings of the brains of a child, two sheep and a dog, in which he figures the blood supply of the brains. Black ('13) figures the encephalic arteries of a case of cyclopia in man, in which he shows that the superior and posterior inferior cerebellar arteries were all quite small and arose from the basilar, while the anterior inferior cerebellar arteries branched in both directions. Black also indicated that the posterior portion of the cerebral vesicle on the left side was supplied wholly by the posterior cerebral branch of the basilar artery.

As in uncomplicated hydrocephalus (Blackburn, '07, 07a) the blood vessels may be present in normal position, although quite small, as instanced in several cases including one described by the writer ('23).

*Histological structure:* Microscopical study of some of the brains serves to aid considerably in the settlement of questions regarding the fiber tracts and nerve connections. One of the most interesting studies thus made is that of Black ('13).

Duckworth ('08) studied the spinal cord of a defective pig in which the brain was perfectly symmetrical, consisting of medulla, pons and cerebellum, with the structures connected with those parts. The cerebellum capped the pons as in a specimen recently described (Chidester, '14). The tracts of the cord were shown as clearly as in the control and Duckworth thinks that this indicates a strong support for the theory of the laying down of the

pyramidal tracts in situ. In most cases of cyclopia the descending tracts are absent.

### Deformities Other Than of the Head Accompanying Cyclopia

As previously shown, malformations of the head, such as otocephalus, hydrocephalus, anencephalus, hare-lip, absence of either of the jaws, or the presence of the so-called proboscis, are frequent in cases of cyclopia.

Deformities of other parts of the body may occur in association with cyclopia. Some of these, such as polydactyly or club foot, seem to be inherited.

It is worthy of note that deformities in the median line are of frequent occurrence. There may be an absence of one of the paired visceral organs; or there may be inversion of the viscera (Taruffi and Vogt, '94). Spina bifida is common (Panum, '80). Polydactyly seems to be an exceedingly frequent concomitant with cyclopia (Earl, '00). Exomphalus and umbilical hernia have been reported. Diaphragmatic and abdominal hernias are fairly common (Ballantyne, '04). The reproductive organs in the female cyclops are frequently malformed, there being such deformities as uterus bicornis duplex and septate vagina (Renner, '89).

### Experimental Production of Cyclopia in the Lower Vertebrates

A number of the early workers on the development of the egg of the common fowl secured occasional cases of cyclopia by varying the temperature of the incubator, shaking, varnishing, electrical stimuli, reducing the supply of oxygen, etc. Experimental teratogenesis established amniotic defects as the most probable cause of the occurrence of the monstrosities and it was the belief of Dareste that cyclopia was due to the arrest of development of the anterior cerebral vesicle, the result of the premature closure of the cerebro-spinal groove.

Féré ('93–'03) not only repeated the experiments of

Dareste and others, altering temperature, traumatism, mechanical vibration, colored light and varnishing, but he tried the effects of gases which might penetrate the shell, surrounding the eggs with an atmosphere of turpentine, musk, mercury, phosphorus, ammonia, ether, chloroform, alcohol and tobacco, before incubation. He also injected substances in solution into the albumen of the egg. He used solutions of salt, glucose, glycerine, potassium iodide, potassium and strontium bromide, peptone, creatinine, xanthocreatinine, cantharidine, nicotine, sulphate of strychnine, many alcohols, acetone, toxins, blood, morphine, atrophine, atropine, cocaine and venine. In all Féré's work, control experiments were made and large quantities of eggs were used.

Féré secured omphalocephaly, atrophy of the head, double heart, atrophy of the tail, cyclopia and other monstrosities by means of almost all the injected substances; and secured a large quantity of monsters by the subjection of the eggs to gases and by agitation. He found that the intensity and duration of action of the disturbance caused a greater number of monsters, but he did not find that any specific influence was responsible for a particular monstrosity.

The introduction of anesthetics and toxins into the eggs by Féré was a long step in advance of any previous work in experimental teratogeny. Dareste decided from his experiments that the arrested development of the embryo was the cause of the defect in the amnion which acted by pressure on the embryo. Féré, on the contrary, showed that the same anomalies may occur both with and without arrest in the development of the amnion. Féré seems to be of the opinion that a change in nutrition is the cause of monsters. In speaking of Féré's work, Ballantyne ('04) states his belief that the two hypotheses may be combined and suggests that in the case of the human ovum, there may be a defect in nutrition which arrests the growth of the amnion, leading to pressure of the amnion and subsequent arrest of the development of

the embryo. Ballantyne holds that in cases where the amnion is not arrested and the embryo is malformed, the general cause, nutritional or otherwise, came into action after the amnion had been formed, and acted in some other way on the embryo.

Born ('97) secured cyclopia in the frog by splitting the head sagitally after the formation of the medullary plate and then readjusting the halves.

Spemann ('04) ligatured salamander eggs obliquely to the first plane of cleavage and secured embryos with double heads, one of which was frequently cyclopean.

Lewis, in 1905, secured cyclopean Funduli by pricking the anterior end of the embryonic shield. In many cases the prick destroyed the nose only. Mall, who reports the work ('08), holds that this shows that it is the absence of the tissues that allows them to come together and unite, and that a rudimentary brain is unnecessary in cyclopia. (See Lewis, '09.)

In 1907 Harrison produced cyclopia by the entire removal of the brain in frog embryos. (Mall, '08.)

Stockard found ('07) that the eggs of Fundulus developing in a solution of $MgCl_2$ in sea water developed cyclopic deformities. Later ('09) he advanced the theory that the action of the magnesium was anesthetic, inhibiting the optic outpushings. Later work by the same author ('10) showed that alcohol and other anesthetics produce a large per cent. of cyclopean and monophthalmic Funduli. Chloretone, chloroform and ether are more general in their action but many cyclopean fish are produced by them. Stockard's work served to show that normal eggs may be caused to develop into cyclopean monsters by chemical disturbance. He has also carried out experiments on the eggs of the fowl and produced deformities in chicks as did Féré.

McClendon showed conclusively ('12) that cyclopean monsters may be produced in Fundulus by many different substances such as KCl, NaCl, NaOH and cane sugar, thus disproving earlier beliefs in the specificity of sub-

stances such as salts and anesthetics. Newman, who had previously secured various types of monstrosities by the *hybridization* of fishes (1908 et seq.), suggested in 1917 that the *axial gradient* theory of Child, "a quantitative one, depending on the rate of metabolic activity, is the true explanation of teratological development in axiate forms." The anomalies included numerous types of defects of eyes and ears. It is worthy of note that the "axial gradient" theory, long unrecognized, is now being accepted pretty generally as the explanation of varying anomalies. (Child, "Individuality in Organisms.")

Loeb (1915) showed that eye defects result in fishes reared in darkness and under low temperature conditions.

Werber ('15) produced cyclopic Funduli by means of butyric acid and acetone and suggested that monstrous development was due to metabolic toxemia.

Kellicott ('16) showed that low temperature produced abnormal development, including cyclopic deformities, in the fertilized eggs of Fundulus heteroclitus. He concluded that the cause of the abnormal development was a disturbance of the normal organization of the ovum, and suggested his "disorganization" hypothesis in place of the nutrition and metabolism hypothesis of Mall, Stockard, Werber and Loeb.

Gladstone and Wakely ('20, '23) incline also to the belief that cyclopia and agnathia, like hare-lip and cleft palate, are attributable to defective nutrition at the proper stages.

Whether there may not also be a *germinal factor* in some cases of the cyclopic deformity is not yet shown. Wilder ('08) inclines to the belief that there is such a germinal factor.

It was shown in a most striking way in the case of monophthalmia in one of twin fishes described by the writer (Chidester, '14), that an anesthetic may produce a defect of the eyes in one of identical twins, without in any way injuring the other fish. If the defect had been a germinal one, it is likely that the identical twins would

have both been abnormal. The not infrequent occurrence of identical deformities in mammalian twins may of course be readily explained on the basis of nutritional disturbance and to that idea the writer is quite ready to subscribe.

Child ('11) discovered in 1909 that he could produce cyclopia in the Plathyhelminth, Planaria by means of anesthetics such as chloroform, alcohol, chloretone and ether, and it was due to his suggestion that the writer engaged in the use of these substances in connection with Fundulus experiments. All gradations of the monophthalmic and cyclopic order were produced with ease in these remarkable experimental animals so long used by Dr. Child.

Since we have suggested the theories of possible origin of cyclopia, it will be well to take up some of the older theories and elaborate upon them.

## The Origin of Cyclopean Monsters

The history of the conjectures regarding the origin of cyclopean monsters is essentially the same as that of the origin of other teratoma.

The early theories of the supernatural origin of monsters were followed by theories based on an incomplete knowledge of the parts played by the semen, menses and ova in the development of the embryo.

Hybridity was held to be the cause of monsters until Bianchi, in 1741, pointed out that when the product of human gestation had a rough resemblance to an animal form, this resemblance might be due to a fetal disease or the product itself might be a mole or retained portion of the placenta. Duval advanced the theory that sterility between animals of different species may be due, not to an absence of impregnation, but to such an abnormal development subsequent to impregnation as to cause arrest by its very abnormality.

Passing over the theories which are not tenable in the light of our present knowledge of pathology, we may

profitably consider a few of the more plausible explanations of the origin of mammalian cyclopia.[2]

### (*a*) *Mechanical Theory*

Dareste held ('77) that cyclopia was caused by the compressing action of the head folds of the amnion.

Mall ('08), from an extensive study of early human embryos, has come to the conclusion that pathological embryos are due to the faulty implantation of the ovum, resulting in faulty nutrition.

Hannover (Ballantyne, '04) believes that in cyclopia the primary defect is due to an imperfect formation of the prechordial part of the base of the skull.

### (*b*) *Diseases of the Foetus*

With respect to the foetus, following Ballantyne ('02, '04), we may consider (1) diseases of the foetus itself; (2) diseases of the foetal membranes which set up abnormal conditions in the embryo or foetus.

(1) Hydrocephalus was very early attributed as the cause of anencephaly. Beclard, 1816, advanced the theory that dropsy is one of the most frequent diseases of the foetus, and that it might give rise to a hydroencephalic hernia. He also held that the destruction of the olfactory nerve would cause the atrophy of the ethmoid bone and the union of the two eyes. This for Beclard explains cyclopia, otocephalus and paracephalus.

Bruce ('96) regards as a separate or contributing cause of cyclopia a limited pachy- and lepto-meningitis. Blackburn ('07a) believes that uncomplicated hydrocephalus is caused by an inflammation of the ventricles.

It is possible that in the mammals, where hydrocephalus is a frequent occurrence in cyclopia, hydrocephalus is a contributory cause. Since in the non-cyclopean hydrocephalic brain there are many anomalies of the Circle of Willis (Blackburn, '07), it is exceedingly difficult to

[2] The historical portions of this account are compiled from Ballantyne. ('02, '04).

determine the part which any abnormality of the blood vessels in cyclopean hydrocephalus may have played in the production of the monstrosity. It is hoped that the study of a large number of specimens may throw some light on this point.

(2) In the discussion of the diseases of the foetal membranes as possible causes of monstrosity, the amnion has been the center of attention. Schroeder believes that syphilis may cause hypertrophy of the villi of the chorion.

Amniotic disturbances as a cause of cyclopia has many champions. Dareste holds that cyclopia originates as a result of amniotic compression which causes an arrest of development of the anterior cerebral vesicle. (Dareste, '77, '91) Rabaud ('01), however, showed that in artificially produced cyclopia in the chick, in which there was evidence of amniotic compression, the morbid changes are not those of true cyclopia, but rather of states resembling cebocephaly or approximation of the eyes. Ballantyne ('04) points out that the amniotic influence may be due to disease, poisons or mechanical states, which may delay the formation of the amnion. One strong point against the amnion as a factor in producing cyclopia or any other monstrosity, is the fact that the *formation of the amniotic sac precedes that of the embryo.*

### (*c*) *Maternal Diseases*

If syphilis produces hydrocephalus, it is quite possible that cyclopia is produced by the hydrocephalus and thus indirectly by disease. It is equally possible that other diseases in the mother may cause the inhibition of the development of whatever system is being developed at the time of the onset of the disease.

It is highly probable that disturbances of the functions of the *organs of internal secretion* are responsible for many of the foetal monstrosities.

### (*d*) *Pathology of the Germ*

In the light of the work of Bardeen ('07, '11) and

others, we must admit that a teratogenic condition may be produced in the embryo by subjecting the ova or the spermatozoa to external agents. It is not a very great step to the supposition that there may be an inherited teratogenic condition of the blood which is the cause of the malformed development of the embryo, and it is even less hard to believe, in the light of what we know of the occurrence of the same malformations in several generations of the same family, that the teratogenic conditions may exist in the germinal or preembryonic state, before fertilization. The causes of these teratogenic conditions may be mechanical, chemical or toxic. In the discussion of experimental work done upon various groups of animals, it is often forgotten that after the stimulus of the spermatozoon, the development is truly subject to the influences of environment, whether the embryo be in a shell outside the body of the animal, or whether the development takes place in the body of a mammal, with an amnion and a placenta.

It might be profitable to consider a few of the cases in which in mammals a possible predisposition—germinal factor if you will—caused the birth of more than one cyclopean monster to the same mother.

Caradec recorded a case in which a woman gave birth to a female cyclops, then to three well-formed males, and then to three females, of which one had hydrocephalus, the second hare-lip and cleft palate, and the third cyclopia (Ballantyne, '04). Brotherston recorded a cyclopean lamb which was one of thirty similarly deformed, all the offspring of one ram (Symington & Woodhead, '88).

It is possible that all the cases recorded in man may be traced to similar methods of living during the early stages of pregnancy, or it is of course possible that either *ova* or *spermatozoa* were defective. We do not know in the case of the cyclopean lambs whether there were more than two or three females which gave birth to the cyclopean lambs sired by the same ram. We know nothing of the conditions of grazing of the flocks, existence of pos-

sible abortifacient weeds, or, what may be even more to the point, the relationship of the females who bore the monsters.

It is not necessary to dwell in this connection on the other monstrosities which are born in succession to the same parent. Suffice it to say, certain fish were found by Born ('97) to be prone to yield double monsters, the tendency remaining almost constant and but little influenced by a difference in the source of the spermatozoa. Féré found that some hens were prone to lay double yolked eggs. Stockard's legless lambs ('07 and '09) were sired by the same ram and one of the mothers bore two legless lambs at the interval of a year. Stockard holds ('09) that in this flock the occurrence "is probably due to germinal variations, either within the mother or father or both." In monsters it is well known that the female sex predominates. Possibly the same factor which causes the mammalian ovum to develop into a monster may stimulate the action of a sex determiner. Present chromosome theories do not admit this possibility.

### (*e*) *Drugs and Anesthetics*

Porak has shown ('94) that mercury, phosphorus and arsenic may cause abortion in the guinea pig. Arsenic is a particularly strong abortifacient, causing placental hemorrhages. Lead does not produce abortion. Observations have been made by various scientists on the offspring of workers in mercury, lead, phosphorus and arsenic and the general conclusions seem to be that they act similarly to syphilis, in producing abortions, malformations of the brain and visceral lesions. There is apparently paternal as well as maternal transmission. No cases of cyclopia have been directly traced to these causes.

It has been clearly shown by Nicloux ('08) that in guinea pigs, ether, chloroform and alcohol can pass from mother to foetus. The effects of these anesthetics on the chick and the fish have been previously discussed. For

centuries, monstrosities have been attributed to the drunkenness of the parents. Early work was largely conjectural, and based on statistics, which were frequently manipulated to suit a preconceived idea. More recent statistical work, such as that of Sullivan and of Pearson, shows a little better science, but neither of these workers has proved his point.

Mairet and Combemale ('88) secured from an alcoholized bitch seven puppies, four of which were dead, two healthy with but little intelligence and the last physically and mentally backward. The offspring from this animal when bred with a normal male were deformed. One had clubfoot, another had muscular atrophy of the hind legs.

Palazzi, on the contrary, found ('01) that in rabbits injected with alcohol during pregnancy, the five which became pregnant, of seventeen in all, gave birth to twenty-four fetuses all of them living and well formed.

Hodge found ('03) that the offspring of alcoholized dogs were deformed and non-viable.

Laitinen found ('08) that the offspring of alcoholized guinea pigs and rabbits were frequently stillborn.

Nice, feeding alcohol, nicotine and caffeine to white mice and subjecting others to the fumes of tobacco, found ('12) that of 707, none were deformed nor born dead. The young of the alcoholized mice weighed more than the others.

Stockard ('12, '13, '16) has shown that in guinea pigs it is possible to induce the appearance of deformities among the young of parents subjected to the fumes of alcohol daily for six days per week. He secured various stages of eye-defects from anophthalmia to monophthalmia. The descendants of some of the young were in enough cases defective to indicate that there had been an injury to the germ cells.

## SUMMARY

(1) Cyclopia occurs in mammals, birds and fishes.

(2) The mammalian cyclops does not live long on account of inability to breathe.

(3) All gradations from a single median eye to an hour-glass eye and finally to two eyes close together are classed as of the cyclopean order.

(4) The optic nerves present corresponding degrees of abnormality, there being sometimes no nerves, again perhaps a single nerve, and finally two nerves forming a more or less complete chiasma.

(5) The internal recti muscles are generally absent. The obliques may be present. The external recti are generally present.

(6) The nasal proboscis frequently accompanies cyclopia. It is fleshy and is supposed to be the fleshy part of the nose, the cartilaginous part of which is prevented by the median eye from assuming its normal position.

(7) In mammals, cyclopia frequently occurs with hydrocephalus, the skull is greatly distorted. In cyclopia unaccompanied by hydrocephalus, the frontal bones are fused; the ethmoid, nasal, superior maxillary, lachrymal, vomer and inferior turbinated are absent.

(8) The first cerebral vesicle is the one most affected in cyclopia. The cerebrum is frequently a single vesicle with the lateral ventricles fused with the third. The corpora quadrigemina come from the mesencephalon and are generally normal. The pons, medulla, cerebellum and fourth ventricle are usually normal. All the cranial nerves back of the fourth are generally normal. The Circle of Willis may be normal.

(9) Deformities of parts of the body other than the head are frequent accompaniments of cyclopia. The malformations most frequently occurring with cyclopia are spina bifida, polydactyly, inversion of the viscera and abdominal hernia.

(10) Cyclopia has been produced mechanically in the flatworm, fish, amphibian and bird; by hybridization in the fish; by temperature change in the fish and the bird; and chemically in the flatworm, fish, bird (chick) and mammal.

(11) Cyclopia in mammals, as in lower forms, is due to

the action of some physical or chemical agent at the period just before the optic vesicles are pushed out. The extreme susceptibility to external influence manifested by the developing sense organs, particularly the eyes, renders deformities of the cyclopic order of quite surprising frequency in experiments with lower vertebrates, and the theory is advanced that disturbance of the metabolism of the mother due to disordered secretion of the hormones may be a contributing cause of numerous mammalian deformities.

## BIBLIOGRAPHY

Ahlfeld, F.
1880. Die Missbildungen des Menschen. Leipzig.

Allan, R.
1848. Dissection of a human astomatous cyclops. Lancet, Vol. 1, p. 227.

Baldwin, W. M.
1919. The artificial production of monsters conforming to a definite type by means of x-rays. *Anat. Rec.*, Vol. 17, No. 3, pp. 135–163.

Ballantyne, J. W.
Manuals of Antenatal Pathology. Edinburgh. 1902. Vol. 1. The Foetus. 1904. Vol. 2. The Embryo.

Banchi, A.
1905. Del Cranio e del Cervello di dui Ciclopi. Archivio di Biol. norm. patol. A. 9, fasc. 2. April, 1905.

Bardeen, C. R.
1907. Abnormal development of toad ova fertilized by spermatozoa exposed to Roentgen rays. *Jour. Exp. Zool.*, Vol. 4, p. 1.
1911. Further studies on the variation in susceptibility of amphibian ova to the x-rays at different stages of development. *Am. Jour. Anat.* Vol. 11, pp. 419–495.

Black, D. D.
1913. The central nervous system in a case of cyclopia in homo. *Jour. Comp. Neurol.* Vol. 23, pp. 193–257.

Blackburn, I. W.
1907. Anomalies of the encephalic arteries in the insane. *Jour. Comp. Neur. and Ps.*, Vol. 17, Nov. 1907.
1907a. Hydrocephalus. Washington Medical Annals.

Born, G.
1897. Ueber Verwachsungsversuche mit Amphibien larven. Archiv. fur Entw. d. Org. Bd. 4, 349, p. 517.

Bruce, A.
1896. On a human cyclops. Proc. Roy. Soc. Edinb. Vol. 20, pp. 412–421.

Chidester, F. E.
1914. Cyclopia in mammals. *Anat. Rec.*, Vol. 8, No. 6, pp. 355–366.
1914a. Twins in fish, one with a cyclopic deformity. *Anat. Rec.*, Vol. 8, No. 6, pp. 367–369.
Child, C. M.
1911. Experimental control of morphogenesis in the regulation of Planaria. *Biol. Bull.* Vol. 20, No. 6, pp. 309–333.
Child, C. M. and McKie, E. V. M.
1911. The central nervous system in teratophthalmic and teratomorphic forms of Planaria dorotocephalia. *Biol. Bull.*, Vol. 22, No. 1, pp. 39–59.
Cleland,
1877. On the brain in cyclopeans. *Jour. of Anat. and Physiol.*, Vol. 12, p. 518.
Dareste, M. C.
1877. Recherches sur le mode de formation de la cyclopie. C. R. Acad. de Sci., Paris. Vol. 84, p. 1038–
1880. Des monstres otocephaliens. T. 90, pp. 191–193.
1891. Mode de formation de la cyclopie. *Ann. d'Oculistique.* 1891. pp. 171–182.
Duval, M.
1881. Sur un monstre otocephale. *Gaz. med de Paris.* 6s. T. 3. p. 255.
Earl, H. C.
1900. Cyclopean foetus with supernumerary fingers and diaphragmatic hernia. Dublin *Quart. Jour.* Vol. 110, pp. 226–227.
Duckworth, W. L. H.
1908. Description of a microcephalous new-born pig in which the face and the fore-parts of the brain were undeveloped and the buccopharyngeal membrane remained imperforate. Proc. Camb. Phil. Soc. Vol. 14, pp. 447–456.
Féré, Ch.
1894–1903. Series of papers on injection of alcohols, essences, nicotine, strychnin and many other anaesthetic and toxic substances, into the egg. Mem. de la Soc. de Biol. 1894. Compt Rendu de la Soc. de Biol. Paris, 1894–1903
Gemmill, J. E.
1906. On cyclopia in osseous fishes. Proc. Roy. Soc., London. Vol. 1, pp. 443–449.
Gladstone, R. J.
1910. A cyclops and agnathic lamb. *Brit. Med Jour.* Vol. II. p. 1159, 1910.
Gladstone, R. J. and Wakely, C. P. G.
1920. A Cyclops Lamb (C. Rhinocephalus). *Jour. of Anat.*, Vol. 54, pp. 196–207.
Gladstone, Reginald J. and Wakely, C. P. G.
1923. Defective development of the mandibular arch. The etiology of arrested development and an inquiry into the question of the inheritance of congenital defects. *Jour. of Anat.*, Vol. 57, Part I, pp. 149–167.

Gould, G. M. and Pyle, W. S.
1897. Anomalies and curiosities of medicine. Phila.
Hirst and Piersol.
1892. Human monstrosities. Phila.
Hodge, C. F.
1903. The influence of alcohol on growth and development. Physiol. aspects of the liquor problem. Vol. I, pp. 357–375.
Jenkinson, J. W.
1909. Experimental embryology. Oxford.
Kellicott, W. E.
1916. The effects of low temperature upon the development of Fundulus. *Am. Jour. Anat.*, Vol. 20, pp. 449–482.
Laitinen, T.
1908. Ueber die Einwirkung der kleinsten Alkoholmengen auf die Widerstandsfähigkeit der tierischen Organismus mit besonderer Berucksichtigung auf die Nachkommenschaft. Zeitsch. für Hygiene. Bd. 58, pp. 139–164.
Lewis, W. H.
1909. The experimental production of cyclopia in the fish embryo (Fundulus heteroclitus). *Anat. Rec.*, Vol. 3, pp. 175–181.
de Lima, J. A. Pires.
1918. Study of an opodymous kitten. *Jour. Anat. and Physiol.*, Vol. 52.
1920. Anatomy of a fetus of a cyclopean goat. *Anat. Rec.*, Vol. 19, No. 2, pp. 73–81.
Loeb, J.
1915. The blindness of the cave fauna and the artificial production of blind fish embryos by heterogeneous hybridization and by low temperatures. *Biol. Bull.*, Vol. 29, pp. 50–68.
Mairet, A.
et Combemale.
1888. Influence degenerative de l'alcool sur la descendance. Recherches experim. C. R. de Soc. de l'Acad. des Sc. Paris. T. 106, pp. 667–670.
McClendon, J. F.
1912. An attempt towards the physical chemistry of one-eyed monstrosities. *Am. Jour. Physiol.*, Vol. 29, pp. 289–297.
Mall, F. P.
1908. A study of the causes underlying the origin of human monsters. *Jour. Morphol.*, Vol. 19, pp. 3–368.
Moenkhaus, W. J.
1910. Cross fertilization among fishes. Proc. Indiana Acad. Sci., 1910 (1911).
Naegeli, O.
1897. Ueber eine neue mit Cyclopie verknüpfte Missb. des Centralnervensystems. Archiv. f. Entw., Bd. 5, pp. 169–218.
Newman, H. H.
1908. The process of heredity as exhibited in the development of Fundulus hybrids. *Jour. Exp. Zool.*, Vol. 5, no. 4.

1914. Modes of development in teleost hybrids. *Jour. Exp. Zool.*, Vol. 16, pp. 447–490.

1915. Development and heredity in heterogenic hybrids. *Jour. Exp. Zool.*, Vol. 18, no. 4, pp. 511–576.

1917. On the production of monsters by hybridization. *Biol. Bull.*, Vol. 32, no. 5, pp. 306–321.

Nice, L. B.

1912. Comparative studies on the effects of alcohol, nicotine, tobacco smoke and caffeine on white mice. I. Effects on reproduction and growth. *Jour. Exp. Zool.*, Vol. 12, pp. 133–152.

Nicloux, M.

1900. Passage de l'alcool ingere de la mere au foetus. L'Obstetrique. T. 99.

1906. Passage du chloroform de la mere au foetus. C. R. de Soc. de Biol. T. 60, pp. 373–375.

1908. Passage de l'ether de la mere au foetus. C. R. de Soc. de Biol. T. 64, pp. 329–331.

Paolucci, L.

1874. Sopra una forma monstruosa della Myliobatus noctula. Atti della Soc. Ital. di Sc. T. 17, pp. 60–63.

Palazzi.

1901. Ann. di. ostet e ginec. T. 23, p. 357.

Panum.

1880. Entstehung d. Missbildungen. Berlin.

Phisalix, C.

1889. Monstres Cyclopes. *Jour. d. l'Anat. et de la Physiol.* T. 25, p. 67–105.

Porak.

1894. Archiv. de Med. Exper. et d'Anat. Path. Reviewed in Teratologia.

Rabaud, E.

1900. Les formations hypophysaires chez les cyclopes. C. R. Soc. de Biol. T. 52, pp. 692–694.

1901. Formation de l'oeil de cyclopes. C. R. de Soc. de Biol. T. 53, pp. 238–240.

Renner, C.

1889. Ueber einen Fall von Cyklopie, Polydactylie, Uterus bicornis duplex, und vagina septa. Inaug. diss. Halle a Strasse. 35 p.

Smith, R. M. and Parker, A. J.

1882. Dissection of a human octocephalic cyclops monstrosity. *Am. Jour. Med. Sc.*, Vol. 84, pp. 132–140.

Stockard, C. R.

1907. The artificial production of a single median cyclopean eye in the fish embryo by means of sea water solutions of $MgCl_2$. Archiv. f. Entw. Bd. 23, pp. 249–258.

1909. The development of artificially produced cyclopean fish, "the magnesium embryo." *Jour. Exp. Zool.*, Vol. 6, pp. 285–338.

1910. The influence of alcohol and other anesthetics on embryonic development. *Am. Jour. Anat.*, Vol. 10, pp. 369–392.

1910. The independent origin and development of the crystalline lens. *Am. Jour. of Anat.*, Vol. 10, No. 3, pp. 393–422.

Stockard, C. R.

1912. An experimental study of racial degeneration in mammals treated with alcohol. Arch. Int. Med., Vol. 10, pp. 369–398.

1913. The effect on the offspring of intoxicating the male parent and the transmissions of the defect to subsequent generations. AM. NAT., Vol. 47, pp. 641–

Stockard, C. R. and Papanicolaou, G.

1916. A further analysis of the hereditary transmission of degeneracy and deformities by the descendants of alcoholized mammals. AM. NAT., Vol. 50, I, pp. 65–88; II, pp. 144–177.

Symington, J. and Woodhead, G. S.

1888. Observations on cyclopia in the human subject and in the lower animals. Proc. Roy. Phys. Soc., Vol. 9, pp. 268–279.

Taruffi, C. and Vogt, H.

1894. Cyclops dirrhinus. Mem. d. Roy. Acad. d. Sc. d. Instit. d. Bologna. T. 8, p. 540.

Werber, E. I.

1915. Experimental studies aiming at the control of defective and monstrous development. *Anat. Rec.*, Vol. 9, no. 7, pp. 529–562.

Wilder, H. H.

1908. The morphology of cosmobia; speculations concerning the significance of certain types of monsters. *Amer. Jour. of Anatomy*, Vol. 8, pp. 355–440.

Wyman, J.

1859. Cyclopism in a pig. *Boston Med. and Surg. Jour.*, Vol. 59, p. 121.

Yung, E.

1901. Monstrosity in a trout. Rev. Suisse Zool., t. 9, pp. 307–313.

Zweifel, P.

1877. Der Uebergang von Chloroform und Salicylsäure in die Placenta. Archiv. f. Gynaekol. Bd. 12, p. 235.

# THE THRESHOLD OF FERTILITY IN RATS AND ITS RELATION TO DIET DEFICIENCY[1]

DONALD MACOMBER, M.D.

In 1920 and 1921, working at the Bussey Institution, we carried out certain experiments with rats to determine the effect of deficient diets on breeding and to see whether we could produce sterility by this method. The work was suggested to us by certain effects of deficient diet as reported by Dr. E. V. McCollum, of Johns Hopkins University. He had found that rats on deficient diets not only failed to make satisfactory growth but often failed to reproduce.

The diets which he used were as a rule quite deficient in one or more important factors and for that reason were hardly comparable to diets which might be taken by animals or human beings in a state of nature. For our purpose it seemed wiser to try to devise deficient diets which should fall in this latter class. With the aid of Dr. McCollum our diets therefore were designed so as to be deficient in a given dietary factor but not so deficient as to interfere with growth. An analysis of the diets is given in Table I.

TABLE I.

COMPOSITION OF DIETS

| Stock Diet. | Normal Diet. |
|---|---|
| Hominy | 33.25 |
| Rolled oats | 33.25 |
| Meat scrap | 22.0 |
| Skim milk powder | 11.0 |
| Salt | 0.5 |

In addition unlimited amounts of special "dog" bread with analysis; Carb. 65 per cent., prot. 15 per cent., Fat 7 per cent., calc. phos. 6.7 per cent., Fibre, etc. 6.3 per cent.

[1] This work was aided by a grant from the Elizabeth Thompson Science Fund.

TABLE I.—COMPOSITION OF DIETS (*Continued*)

| Diet A. | | Low in Calcium. |
|---|---|---|
| Whole wheat | 67.5 | |
| Casein | 15.0 | |
| Whole milk powder | 10.0 | |
| Sodium Chlorid. | 1.0 | |
| Inert substance | 1.5 | |
| Cod liver oil | 5.0 | |
| **Diet B.** | | **Low in the Fat Soluble Factor.** |
| Rolled oats | 40.0 | |
| Gelatin | 10.0 | |
| Casein | 5.0 | |
| Salt mixture (McCollum) | 3.7 | |
| Dextrin | 40.3 | |
| Cod liver oil | 1.0 | |
| **Diet C.** | | **Low in Protein and Calcium.** |
| Wheat whole | 60.0 | |
| Sodium chlorid. | 1.0 | |
| Inert substance | 1.5 | |
| Dextrin | 32.5 | |
| Cod liver oil | 5.0 | |
| **Diet D.** | | **Generally Deficient Diet.** |
| Wheat bran | 20.0 | |
| Wheat middlings | 14.0 | |
| Ground oats | 13.0 | |
| Corn meal | 9.5 | |
| Gluten meal | 10.0 | |
| Meat scrap | 12.0 | |
| Alfalfa meal | 20.0 | |
| Powder chalk | 1.5 | |

The result of these early experiments bore out what we had anticipated and we did actually produce a certain number of completely sterile animals. Post-mortem examination showed that there were no gross anatomical abnormalities, and sections of ovaries and testicles failed to show any noteworthy changes in these organs. We had used for our work a strain of Albino rats which had been inbred by Dr. Helen D. King, of the Wistar Institute, for 33 generations of brother and sister mating. She had found that inbreeding at first much reduced health and fertility, but that if the individuals chosen for continuing the strain were selected for vigor the fer-

tility and health of the stock became restored, though at a somewhat lower level than for controls. In order to accumulate a sufficient number of these rats for our experiments it became necessary to continue the inbreeding. In this way we accumulated records of a good many matings of rats on a normal diet (the so-called "stock diet"). We found that there was the greatest possible difference in the fertility of the various individuals. For instance, the matings of certain individuals were practically invariably successful, whereas in other cases only one or two out of a number might be positive, and there were all degrees of variation between them. We were forced, therefore, at the outset to the conclusion that even in this homozygous strain individuals varied as to fertility as well as in other characteristics.

Having accumulated a sufficient stock of young and fully mature animals of this inbred strain we proceeded to submit them to the various diets already referred to in Table I. Our procedure with the adult animals was first to test their fertility, then place them on diet for one month and re-mate. Before a mating was considered negative it was allowed to continue from six weeks to two or more months. The young animals were put upon a diet directly after being weaned and were allowed to grow to maturity upon it before being mated. In our first experiments all matings were between rats which had been submitted to the same diet. When we came to check up results it seemed that we had produced a good deal of sterility, as many of the matings so made up were negative. In order to be certain of our ground, however, we first re-mated each "sterile" animal with a highly fertile rat which had been raised and tested on the stock diet. The result of these re-matings in many cases was immediate reproduction. In 37 cases it proved that both of the animals supposed to have become sterile through diet and which were unable to have young together proved to be promptly fertile when re-mated with these new and highly fertile partners. In many other cases one of the original pair proved fertile.

It seemed impossible to explain these results with any knowledge at our command, and we were forced to formulate our hypothesis of relative fertility. The problem was how to explain the fact that two individuals who might have a sterile mating together would promptly reproduce with other partners. They must have been fertile in some degree all the time, but apparently their degrees of fertility were not sufficient when combined to produce young, and yet that of each was sufficient for reproduction with a more highly fertile partner. We adopted for trial a hypothesis which in brief was as follows—that the fertility of a mating could be expressed as the product of the fertility of the individuals concerned, and that if this mating fertility were below a certain level which we called the "threshold for reproduction" no young would result, but that if it were above this level the mating would be positive. We advanced this hypothesis in a tentative way, for we realized that we had not sufficient experimental proof to confirm it in any degree, but it seemed sufficiently probable to warrant collecting a large number of breeding records with which to test it out. As controls we had the records of 1,381 rats bred by Dr. Castle on stock diet. They were of many varieties as far as genetic constitution is concerned and were as a whole highly fertile. Out of 1,172 females 1,109 were fertile, 4 were completely sterile, 15 doubtful and 44 were killed or died from disease or other cause. Of 209 males 190 were fertile, 7 sterile, 9 doubtful and 3 died or were killed. The fertility of this stock was therefore very high and is to be contrasted with that of the inbred rats where the percentage of sterile and doubtful cases was very much higher. As stated above, the effect of diet upon these inbred animals had been still further to reduce fertility and to increase the amount of sterility. We have records of 622 matings of these inbred rats. Some of these matings were between rats whose fertility had been artificially lowered by deficient diet, some were between inbreds naturally of lowered fertility, and some

with proved animals from the control stock; thus every possible combination was made in our matings. These records are given in condensed form as an appendix to this paper. An analysis of them shows that there were 46 rats which were mated 5 or more times; 19, 4 times; 33, 3 times, and 42 with only 1 or 2 matings each. If we for the moment disregard all those rats which had less than 5 matings, and classify these 46 animals according to the percentage of successful matings which each had, we can get perhaps a rough indication of their comparative fertilities. There were only 2 where 80 per cent. or better of the matings were successful; in 5, between 60 and 80 per cent. were successful; in 10, 40 to 60 per cent.; in 10 more, 20 to 40 per cent., and in 5, less than 20 per cent. In addition to these 13 other animals proved to be completely sterile. This immediately gives us a rough method of classifying the fertility of these individuals, but it will of course give a false picture to this extent, namely, that the other partner of each mating must be taken into consideration, and that a negative mating with a rat all of whose matings are equally negative should not be counted as necessarily detracting from the fertility of the first individual. In other words, the breeding record of every individual must be considered in conjunction with that of the individual with which he has been mated, and these latter in turn with the records of those other rats with which they have been mated. It will be seen that it is possible by a method of trial and error and repeated revaluation gradually to approximate figures which will represent the degree of fertility for all individuals concerned. It is convenient to make these estimations as percentages, and when fertilities are so estimated it is found that in practice the figures so obtained can be used to calculate the fertility of a mating and to predict whether it will be a productive or an unproductive one.

The manner in which the breeding records (see the appendix) were used to calculate the approximate fertilities

of the individual rats was, as has been said, by a process of comparison and constant revaluation. A few examples will give the method by which these estimated fertilities were derived. First, let us take the record of rat No. 1. He was mated with 3, 80, 5, 24, 26, 68 and 32F. All the matings were positive with the exception of the one with 24. Rat No. 1, therefore, is obviously of high fertility, but the degree of fertility can not be calculated without consulting the records of all these animals, for it might be that, with the exception of 24, they were all of very high fertility, in which case we would get no help as to the degree of fertility of No. 1. Similarly, it would influence our judgment as to No. 1 to find that 24 was of reasonably high fertility or the reverse. A glance at the records of 3, 80, 5, 26, 68 and 32F shows them to be of varying fertility, some, such as 68 and 32F, are of rather low fertility, while 3 and 5 are of average fertility. Since all their matings with No. 1 were successful that makes our estimate of No. 1's fertility all the higher. The record of 24 is poor (see appendix); in fact so poor as not to detract materially from our estimate of No. 1. We are safe, then, in considering No. 1 to have a fertility between 90 and 100 per cent. One or two other examples will suffice. Rat No. 1, as indicated by the letter "s," was on stock diet. Rat No. 17 was a male on diet C. After having been on the diet for a sufficient period of time it was mated with 13, which had been on the same diet. Their mating was unsuccessful. They were each then mated with animals of proved high fertility from Dr. Castle's vigorous strain, which had been on stock diet. Rat 13 never had a successful mating, but 17 did have 1 positive mating out of 5 such re-matings. The fertility of 13 can not be definitely assigned, but it is certain that it was much lowered and the probability is that it was so low as to render this rat physiologically sterile. In the case of 17 it is obvious that there was some degree of fertility. It is difficult to make an accurate estimate, however, until we have discussed further results and

used our theory as to the threshold value for reproduction.

Proceeding in the manner which has just been indicated, and with constant reference back to the actual breeding records of the various rats, it was possible in many cases to give approximate figures representing the individual fertility of these rats. The next step was to substitute the values so deduced in our mating formula. We had already been able to calculate approximate values for the fertility of individuals, but we now wished to be able to assign a value to the fertility of a mating. This mating fertility we postulated as the product of the individual fertilities of the two partners. We chose this formula in preference to one derived from the sum or the average of the individual fertilities, since it comes nearer to expressing the truth of the actual results and since it is the only formula which gives practical aid in calculating individual fertilities and predicting results. It must of course be understood, however, that at this stage of our knowledge such an assumption is quite arbitrary. Putting the formula into mathematical shape, it becomes $x \times y = m$, where x is the fertility of the male, y of the female, and m of the mating, all expressed in percentage form. When the calculated values for x and y are substituted in this formula a simple multiplication of the two gives us the fertility figure for that particular mating. This procedure was carried out wherever the individual fertilities were known, and the values for the mating fertilities so derived were arranged in a series. It was at once discovered that all the values for those matings which had been productive were above a certain figure, and the values of those which were negative were below this figure. This figure proved to be in the neighborhood of .50 or 50 per cent. We gave it the name of the Threshold for Reproduction. There were, however, certain exceptions to the general rule, but it was found that they were grouped closely about this level. In order to explain these anomalous cases we put forward the

suggestion that instead of a rigidly fixed value, such as 50 per cent. for the Threshold, there is rather a doubtful zone extending above and below it. With this change we can now state definitely that all matings above the doubtful zone were positive, and all matings below were negative. With our present rough methods of estimating individual fertilities it is impossible to predict certainly whether a given mating will be positive or negative if it falls within this zone.

If this theory of the threshold value for fertility is accepted as a working hypothesis it proves to be of the greatest help in further correcting some of the estimations of individual fertilities. As an example of this practical utility, let us consider the records of rats 72 and 73 (see appendix). When they were mated together no young were produced, but when each was remated with a partner of proved high fertility reproduction occurred at once. In making use of our formula let us first assume that 72 and 73 were of equal fertility. If subsequent matings make it probable that this was not the case it will be very simple then to make the necessary changes. Since x equals y, which is the mathematical way of recording our assumption of equal fertilities, the formula become $x \times x = m$, and the figure for m must have been below 50 since no young were produced. Solving this equation, x equals the square root of a number less than 50, or x is less than .70. This figure could not, however, have been less than .50 because in that case the re-matings with 100 per cent. fertile animals would have been negative, whereas we have seen that they were positive. Then, on the assumption that their fertilities were equal, the individual fertility of each becomes somewhere between .50 and .70, and would probably be in the neighborhood of .60; but a glance at their subsequent mating records makes it evident that their fertilities were not equal since after being mated together 72 had 2 other positive matings, while 73 had 2 negatives and 2 positives. From this record 72 must be materially

more fertile than 73. The product of their mating fertidities can not exceed .50 because if it had their original mating would have been positive. On the assumption that they were of equal fertility we assumed a figure in the neighborhood of .60 more or less. If we give a higher value to 72, 73 must be therefore correspondingly less and yet the value for 73 can never be below .50, because if it were the animal would have been permanently sterile. A figure for 73 somewhere between .50 and .60, say .55, is probably not far from the truth. This would give a figure for 72 of about .65 since their product must not exceed .50.

No further attempt will be made in this paper to elaborate the application of this theory of the Threshold Value for Reproduction to the practical assigning of values for the fertility of individual animals from their breeding records, but enough has been said to indicate the method and to suggest its utility when applied in practice. By its use it will be seen that if the individual fertilities of two animals have been calculated from previous breeding experiments the result of their mating together can be predicted. For instance, the fertility of 99 from its first 6 breeding records can be calculated to be in the neighborhood of .55; similarly, that of 100 to be about the same figure. It could be confidently predicted that a mating between these two would be completely sterile and such proved to be the case. Similarly for the mating between 99 and 108. In the breeding tables in the appendix are given complete records and there also may be found estimated values for the individual fertilities where it was possible to obtain them.[2]

[2] There are several variations of the mating formula $x \times y = m$ which are useful in determining values for either x or y. Where x and y are equal the formula becomes $x^2 = m$, or $x = \sqrt{m}$ and where either x or y stands for the fertility of a proved 100 per cent. fertile animal the formula becomes either $x$ or $y = m$. In making calculations it is usually simpler to assume that the Threshold is .50, hence if a given mating has been productive the value for m must have been higher than .50 and conversely if it has been negative the value will have been below .50. These considerations are often extremely useful in calculating values for individual fertilities. It will be seen of course that they are in no way to be interpreted as having a high degree of mathematical exactness.

These estimated values have been gathered together in Table II and arranged in five columns. The first column is made up from the 1,300 odd breeding records furnished us by Dr. Castle as controls. These were between vigorous animals raised and mated on stock diet. Their genetic constitution was of the most varied type. The second column is made up of all the inbred Albino rats on stock diet of which we had definite records. The last three columns show the effect of various deficient diets upon the fertility distribution among the inbred rats. The third column is for adults which had been raised to maturity on stock diet and whose fertility had already been proved before they were submitted to the deficient diets. The fourth and fifth columns show the distribution for young inbred Albino rats which were allowed to grow to maturity upon the deficient diets (see Table 1—Diets A, B, C and D). As will be noted the control animals are recorded by totals, only whereas the experimental number of each of the other rats is given. These numbers are arranged on a fertility scale from 100 per cent. down to zero. The small letters after each number designate the particular diet to which that rat was submitted, and an analysis of these diets is given in Table I.

Study of this table (Table II) brings out several interesting facts. The typical fertility distribution for highly fertile animals on a normal diet is shown in the first column. It will be observed that a good 95 per cent. are in the most fertile class; that there are a few animals which are of lesser degrees of fertility and a few more which are sterile.[3]

Column 2, while showing a somewhat similar distribution to the preceding, has more animals which are relatively less fertile. The series is too small to be able to decide on the percentage of actual sterility developed by inbreeding; furthermore when one considers the method

[3] Post-mortem examinations of these 10 sterile rats showed nothing abnormal except pyometra in 1 female and pus in the seminal vesicles in 1 male.

TABLE II

| | Fert. Distrib. Castle Rats On Stock Diet | Fert. Distrib. King Rats on Stock Diet | Fert. Distrib. of fertile adults on Defic. Diets | Fert. Distrib. of growing King Rats on Defic. Diet | Fert. Distrib. of growing King Rats on special Defic. Diet |
|---|---|---|---|---|---|
| 100 | | 1, 3, 4, 5, 6, 9, 12, 24, 25, 26, 45, 48, 49, 50, 51, 52, 67, 69, 70, 118, 53 | | 87a, 95b | |
| 90 | 1299 individuals | | | | |
| | | 66, 80 | 3b | 35b, 54b, 57a, 62a, 72b, 97b, 58b, 92a | 122, 123, 125 130 132, 137 |
| 80 | | | | | |
| | 1 individual | | 4b, 5b, 26b, 67b, 25b, 48a? | 27b, 36b, 37b, 71b, 74b, 75b, 77b, 83c, 84c, 86c, 93a, 94b, 104a, 59b | 94, 124, 128 |
| 70 | | | | | |
| | | 44, 63, 64, 68 | 6c, 12c | 16a, 61c, 76a, 78a, 101a, 103a, 105a | 131 |
| 60 | | | 9c? | | |
| | 8 individuals | 43 | | 32Fb, 55b, 73b, 88a, 96b, 106b, 107b, 108b, 15c, 21a | 120, 126, 146 |
| 50 | | | | | |
| | 8 individuals | 65, 79 | | 28b, 17c, 85a, 99a, 100b | |
| 40 | | | | | |
| | | 116 | | 13c, 14c, 18c, 19c, 20a, 29a, 30b, 32Ma, 34b, 38a, 39a, 56c, 60c, 81c, 82c, 98b, 102a | 119, 121, 127 129, 136, 143 Effect of generally def. diet on growing inbreds |
| | 10 individuals | | 24b, 50c, 51b, 52c, 69c, 53a | | |
| 0 | | Spontaneous variation in fert. of inbred (King) rats | Effect of Def. diets on fert. inbred adults | Effect of def. diets on Fert. of growing inbreds | |

Numbers are experimental numbers assigned individual rats. When followed by a small letter a special diet is indicated; a stands for Diet A., b stands for Diet B., and c for Diet C.

For the explanation of this table the reader is referred to the text.

by which these animals had been bred (only from those selected for vigor) it will be seen that this will be no true guide as to the effect of inbreeding on the production of sterility. Columns 3, 4 and 5 show that the effect of deficient diets upon fertility is greatly to increase the numbers of relatively infertile and sterile animals and this is true for adult animals as well as for growing animals though to a lesser extent. The effects of the individual diets do not vary materially except that the effect of

Diet C, which is defective in two elements, is much greater than for either A or B, which are defective in only one. The diets used with the rats in column 5 were generally deficient and were not specially designed as were Diets A, B and C. They were made up chiefly of cereal products and were lacking in vitamine content and succulence (see Diet D, Table I, for a typical analysis). The kind of dietary deficiency, therefore, is not important. It seems rather to be the degree, since the greater the deficiency the larger the proportion of sterility. The way in which the diet seems to affect sterility is through its general effect on the health of the individuals. The less the effect on health the less the effect on fertility.

## CONCLUSIONS

There may be great individual variation in fertilities. Such variation is increased by inbreeding and by deficient diet. Where the variation is great the amount of sterility is also great. There are individuals whose fertility is so low that they are unable to reproduce with one another but whose fertility remains sufficiently high to allow immediate and successful reproduction with highly fertile individuals. We have formulated a theory of fertility to explain these facts. It may be summarized as follows: The fertility of a mating is the result of the product of the fertility of the two individuals concerned. If this mating fertility is above a certain level the mating is productive; if not, unproductive. This level we have called the Threshold of Reproduction. A fairly large series of further matings and re-matings has seemed to confirm this theory at least in its general aspects. The subject requires further experimental study and it will be interesting to see whether there is any hereditary factor involved in fertility.

## BIBLIOGRAPHY

Reynolds, Edward, and Macomber, Donald.
Certain Dietary Factors in the Causation of Sterility in Rats. *Am. Jour. of Obstet. & Gyn.*, St. Louis. Vol. 11. No. 4, Oct. 1921; also Reynolds, Edward, and Macomber, Donald. Defective Diet

as a Cause of Sterility. *Jour. A. M. A.*, July 16, 1921, Vol. 77, pp. 169–175.

McCollum, E. V.

The Newer Knowledge of Nutrition, N. Y., Macmillan Co. 1918 and 2nd edition 1922.

King, Helen D.

*Jour. Exper. Zool.*, 1918, xxvi, 335; 1919, xxvii, 1; 1919, xxvii, 29.

## APPENDIX—BREEDING RECORDS REFERRED TO IN TEXT

*Rat No. 1.* Male—mated with No. 3—young; with No. 80—young; with No. 5—young; with No. 24—no young; with No. 26—young; with No. 32—young. His fertility was estimated to be 95 per cent. more or less since 24 was probably sterile.

*Rat No. 3.* Female—mated with No. 1—young; with No. 6—young; with No. 4—young; with No. 65—no young; with No. 52—no young; with a highly fertile "Castle" rat—young. In estimating the fertility of this rat the failures with 65 and 52 must be considered. A reference to their records shows them to be of low fertility, hence 3's fertility is undoubtedly high—perhaps 90 per cent. more or less.

*Rat No. 80.* Was fertile with highly fertile partners but produced no young with less fertile ones. Fertility estimated at 80 per cent. more or less.

Similarly the fertilities of the following rats were estimated from their records: Rat 5 to be 85–90 per cent.; Rat 24—sterile; Rat 26—75–80 per cent.; Rat 68—65 per cent.; and Rat 32—50–60 per cent.

When these estimations are used to check up the record of Rat No. 1, with which they were all at one time mated, they materially help in the estimation of the fertility of that rat (No. 1).

In a similar way the records of the following rats were derived, namely, those of Nos. 13, 17, 72, 73, 99, 100 and 108 (used in the text as illustrations). For instance, No. 17, a male, was mated with a female, No. 13, without young; but when he was re-mated with highly fertile females young were produced in one out of six matings (fertility 40–45 per cent.); but No. 13 did not produce young though mated twice with highly fertile males (fertility below 40 per cent., *i.e.*, sterile).

Again, No. 72, a female, had no young when mated with No. 73; but produced young with two highly fertile males (fertility 70–80 per cent.), while No. 73 impregnated two out of four highly fertile females (fertility 50–60 per cent.).

Rat No. 99, a male, had negative matings with 104, 100, 108 and three out of five highly fertile females. His fertility was estimated at not over 50 per cent.

Rat No. 100 was a female. She had negative matings with males No. 106 and 99; but two out of four matings with highly fertile males were successful. Her fertility was also estimated at 50 per cent. or slightly more.

Rat No. 108 was also a female. She had negative matings with 94 and two highly fertile males. Her matings with two other highly fertile males were positive; and therefore her fertility was estimated to be about 50 per cent. It could be predicted that a mating with No. 99 (also 50 per cent.) would be negative then, and such proved to be the case.

# THE EFFECTS OF ALCOHOL FUMES ON THE ALBINO RAT: INTRODUCTION AND STERILITY DATA FOR THE FIRST TREATED GENERATION

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## THE PROBLEM

One of the outstanding problems of genetics is that of the origin of new heritable variations. With the passage of time, and the accumulation of exact experimental data, it becomes increasingly clear that this factor is the basic one in evolutionary change, whether progressive or retrogressive.—Pearl, 1917.

There is no a priori reason why the substances of the germ cells should not be as easily altered as any other mass of chemicals. But in practice it turns out that most agents which produce chemical alterations of the germ cells at the same time kill the organism. Further, the germ cells, like other living substances, have a great tendency to compensate for any disturbances induced in them, their condition is one of stable equilibrium, in which an alteration is followed by a return to the original condition.—Jennings, 1920.

SEVERAL investigators have attempted to modify the germ cells of mammals and birds by the administration of daily inhalations of alcoholic fumes, ether and other toxic substances. The papers by Stockard ('16, '18) on the guinea pig, Pearl ('16a, '16b, '17) on the domestic fowl, and Cole and Davis ('14) are among the more prominent papers on this subject.

Because of the fundamental character of the problem involved and the conflicting results obtained by two of the workers above mentioned, it seems desirable to repeat this work on a somewhat larger scale, using a different and possibly more favorable animal than has been employed hitherto.

Three aspects of the problem are here considered:

1. *Somatic:* Does the daily administration of alcohol[1] fumes to albino rats from the ages of sixteen days to ma-

[1] The alcohol used in this experiment was 95 per cent. ethyl alcohol. Hereafter it will be referred to merely as alcohol or alcoholic fumes.

turity produce effects in growth that may be quantitatively measured, and so compared with control animals of the same litter, and with the data on body growth found in Donaldson's ('15) "Book of Reference Tables of the Albino Rat"?

2. *Germinal:* Does the daily administration of alcohol fumes to young albino rats produce alterations in the germ plasm, such as would lead to new heritable variations—either in spectacular abnormalities such as Stockard's guinea pigs showed, or in less evident but definitely measurable variations from the normal growth curve?

3. *Mechanism involved:* Are the somatic effects upon treated animals such as to give any indication of the origin or mechanism of germinal changes; and are the alterations in the germinal materials such as to lend themselves to cytological analysis?[2]

The problem as above outlined is not essentially different from that attacked by Pearl and Stockard. The results of the present authors will be presented in this and subsequent papers.

It is believed that the albino rat offers peculiar advantages in studying those non-spectacular changes which apparently were operative in Pearl's material, and which clearly divided his experimental and control material into two groups, the treated group lacking, however, those striking variations in the form of abnormalities as shown in Stockard's work. As we understand the comparative results of the investigations of Pearl and Stockard, this last-mentioned fact was the chief point of difference between them. They both got definite, but different, modifications as a result of the treatment with alcohol.

[2] Seventeen months after the experiment started, several of the two original litters were turned over to Professor Caswell Grave for cytological study. Testes and ovaries from the treated animals, and from their control brothers and sisters, were preserved in Allen's B-15 and Rigaud's formalin bichromate. The results of the microscopical investigation are not yet available, but will be published in a subsequent paper by Professor Grave. However, there was a marked departure from the normal appearance in both ovaries and testes of the alcoholic rats. The gross appearance of the controls was normal in every respect.

Our method of attacking the problem differs from theirs in this respect, that they used only adult animals that had previously produced at least one lot of normal young. This was a precaution well taken, but omitted in this experiment for these reasons: It is very important, in our judgment, to determine what are the effects of alcohol in modifying the germ cells of animals long before they reach sexual maturity, for young germ cells, it may be, are more easily affected. In the case of the rat, the control of one normal litter may be safely dispensed with, for the litter is large and the members may be expected to behave similarly to one another as to fecundity and body weight. Therefore, selecting at random from each litter half the animals for the experiment and half for the control, it may be expected that the experimental animals, had they not been treated with alcohol, would have grown and behaved as did their control brothers and sisters. Hatai, in his work, used this method for obtaining control and experimental animals.[3]

Treating the germ cells of mammals with toxic substances from near the beginning of their postnatal life is a new departure from all work of a similar kind in attempting to modify germ plasm.[4]

## REVIEW OF THE LITERATURE

The literature in this field has been reviewed by both Stockard and Pearl. These papers are recent, well known and easily accessible.

It has seemed advisable, therefore, to omit any extensive review of the literature as such, and to refer to papers when needed in the discussion of the data presented.

## MATERIAL AND METHODS

As mentioned above the albino rat was selected as being an animal well adapted for this work. It breeds

[3] "This method of comparison is best, since as shown by Jackson the variability of body weight within the litter is only about one half that in the total population." (Hatai, S. '15.)

[4] Since our experiment was started the work of McDowell ('21, '22) has appeared.

very rapidly, throws a large litter and in our experience in raising hundreds of these animals there has never occurred an abnormality of any kind. It is a remarkably stable form.

Another reason for selecting the rat is that through the labors of Jackson ('13), and especially Donaldson ('15), we are in possession of a body of quantitative data on the normal growth of this animal, such as is not available for any other mammal. By the use of control brothers and sisters of the same litter, and Donaldson's reference tables, it is possible to measure and compare any somatic effects induced during the growth process.

A small colony of albino rats was secured from the Wistar Institute of Anatomy and Biology on October 1, 1920. The females were nearly all pregnant when received.

The animals are kept in clean, comfortable cages. The diet has been a general one of table scraps, dog biscuit and grain.[5]

One of the Wistar Institute females gave birth to her second litter in her present quarters on November 22, 1920. She had thrown a litter of thirteen a few days after being received here, and the second litter contained ten young. Another of the Wistar Institute females gave birth to a litter of ten young on January 7, 1921. The experiment herein reported is based entirely upon these two litters of ten each and their descendants. The sex ratio in the first litter was five males and five females. In the second there were four males and six females. The eyes opened on the fourteenth day, and all the animals were in a vigorous condition, as evidenced by their activity and appearance.

The animals of the first litter were numbered from one to ten, and henceforth will be known by these numbers.[6]

[5] Some months after the experiment was begun, cooked food was added to the diet. This was made after a Wistar Institute formula, and contained white flour, corn meal, linseed oil meal, mixed grains, salt and milk.

[6] Our method of numbering is a modification of Bell's ('14). As one faces the rat the numbers begin on the outer edge of the rat's right ear. There are five punched places in each ear. Beginning at the reader's left they read 1, 2, 3, 4, 5, on the rat's right ear; and 6, 7, 8, 9, 0, on its left ear. The numbers 3 and 8 are expressed by punches at the tips of the ears.

Those with numbers from six to ten were the treated, the first five served as controls. The second litter was numbered from eleven to twenty, the last five numbers being those of the treated (see text figure 1).

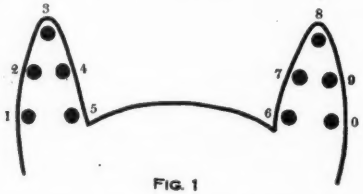


FIG. 1

The rats were weighed individually, to a tenth of a gram, every ten days. The body length and tail length were taken with calipers and these placed upon a millimeter rule to determine the length in millimeters. By a special method of holding the rat the body length was taken in the living animal with very considerable accuracy. All measurements were taken by the senior author with the assistance of a helper in holding the animals.

There was no conscious selection of the animals which were to be controls and those to be treated. The two litters were numbered at random and then divided into two groups, the control group containing numbers one to five and eleven to fifteen, inclusive; the test group with numbers six to ten and sixteen to twenty, inclusive.

TABLE I

A COMPARISON BETWEEN THE CONTROL AND TEST ANIMALS AT THE BEGINNING OF THE EXPERIMENT

| | Body weight (in grams) | | Body length (in mm.) | | Tail length (in mm.) | |
|---|---|---|---|---|---|---|
| | ♂♂ | ♀♀ | ♂♂ | ♀♀ | ♂♂ | ♀♀ |
| Average of controls | 18.54 | 17.22 | 86.10 | 80.50 | 53.70 | 52.70 |
| Average of tests | 18.30 | 17.28 | 85.25 | 86.08 | 54.25 | 53.91 |
| Average differences | — 0.24 | + 0.06 | — 0.85 | + 5.58 | + 0.55 | + 1.21 |

Table I shows that the two groups of animals differ very slightly. The control males are 0.24 of a gram

Any combination of figures can be made by this scheme provided the second or third digits of the number is the same or greater than the first, as reading is always from the left to the right. For example, 32 is not possible, for in reading from left to right it would be read 23.

heavier than the experimental males, while the control females are 0.06 of a gram lighter than their sisters. In body length and tail length the two groups are very close together, with the tests a trifle longer in three out of the four groups. It would be difficult to divide the two litters more equally.

The data are quite convincing on two points: (1) The control and experimental animals at the beginning of the experiment were practically identical, the experimental animals having, on the whole, a slight advantage. (2) These two groups are remarkably normal when compared with similar data from Donaldson's tables on the growth curve in the albino rat.

On December 9, 1920, the animals numbered from 6 to 10 (age sixteen days) were put into an airtight chamber in which 50 cc. of 95 per cent. commercial alcohol, poured on cotton, was evaporating. They remained in the fume tank for thirty minutes, and exhibited all the signs of animals whose mucous membrane was seriously irritated. They rubbed the nostrils and sides of the head vigorously with their front feet. They stood on their hind feet, thus extending their heads as high as possible into the chamber to escape the fumes coming from below. Our observations in this respect agree with those of Pearl and Danforth, for there were no permanent effects produced in the eyes of the treated rats. Towards the end of the treatment period the partial loss of control of the hind legs was noticed. When taken out of the tank the young rats were limp, but in the fresh air recovered rapidly.

On January 25, 1921, the second litter (numbers 16 to 20) were treated for the first time. The conditions of the treatment and the reactions of the animals were the same as reported for the first litter.

The fume tank was made from an old incubator by securely stopping all ventilator openings and stripping the edges of the doors with felt. The interior dimensions of the tank are 12 x 15 x 19 inches. The ten[7] animals could

[7] Male 16 drowned in the water glass on Dec. 26, 1920; and on Sept. 1, 1921, male No. 8 died of pneumonia, after being treated for nine months.

be treated at the same time. Figure 2 is a picture of the fume tank.

FIG. 2

The two litters lived in the same cage with their mothers until they were thirty days old. At that time each litter was given a larger cage, the two cages being alike in size and appointments. Thus controls and alcoholics lived together until they were sixty-two days old, when each rat was put in a separate but similar cage. Every care was taken to see that the environmental conditions of all 20 animals were the same. The single differential between the control and experimental animals is, without doubt, the alcohol treatment.

### Germinal Dosage Index

Certainly the length of time and the severity of intoxication to which the parents have been subjected should have some effect upon the amount of variation, if any, in the offspring. This was recognized in Stockard's papers, but no definite numerical statements of dosage are given. In their last paper Stockard and Papanicoloau ('18) again mention the importance of the amount of dosage as follows:

> Treating female guinea pigs with considerable doses of alcohol fumes only shortly before and during their pregnancies certainly does not injure the offspring to any noticeable degree. While the same degree of treatment, if administered for months or years, will render these mothers almost incapable of producing vigorous young even when mated with normal males.

Pearl has creditably attempted a numerical valuation of the amount of treatment. He calls this valuation "the total germ dosage index." He proposed this term to designate the number of days during which the gametes, making the offspring zygote, have been exposed to the fumes of the toxic substance.

In our experiment no attempt has been made to give a numerical statement of the amount of treatment. The animals were treated daily (Sundays excepted) until a visible state of narcotization was reached. This, of course, involved several increases in the length of treatment and the amount of alcohol. As resistance developed both the time and the amount of alcohol was increased. The rats of the first generation were treated for twelve months, and by that time could withstand four hours with 200 cc of alcohol in the tank.

### Inbreeding

It has long been supposed that close inbreeding is deleterious to stock of any kind, and abnormalities in strains that show a considerable amount of inbreeding are commonly attributed to that fact. Stockard and Pearl give the exact amount of inbreeding present in their strains. From a survey of his stock reaching back four genera-

tions prior to the beginning of the experiment, Pearl concludes that "this stock can not on the average be considered inbred," though it was from a line-bred population, and so to some extent inbred. Stockard and Papanicoloau ('18) state that the alcoholic lines discussed in their paper are practically without inbreeding. Some tables show a slight amount of inbreeding, with no indication of significantly injurious effects. An earlier paper by the same authors seems to show that there were more injurious effects in the alcoholic lines that were inbred than in those that were not.

Castle ('06) inbred Drosophila flies for many generations, his matings all being brother by sister. Fifty-nine generations of this close inbreeding did not injure the fertility of the stock, although there was some reduction in the size of the flies.

Wright ('22) has inbred guinea pigs since 1906, with over 35,000 animals recorded. He reports that "the inbreeding can not be said to have had a specific deleterious effect on general vigor."

Coming to the albino rat, we have a large body of exact data on the effect of one of the closest possible forms of inbreeding (brother-sister within the litter). King ('18a, '18b, '21) has done very exact and conclusive work on the subject. Her experiment has been carried on for over forty generations. "These experiments show that in body growth, in fertility and in general constitutional vigor inbred rats are superior to stock Albinos, reared under similar conditions of environment and nutrition." Miss King's method was brother-sister matings, accompanied by selection.

To summarize: Stockard secured several groups of animals from different dealers and practiced but little inbreeding. Pearl's stock was not essentially inbred, and McDowell's ('21) original stock came from two or more sources.

In the light of the recent work of King, East and Jones, Wright and others, the present authors, instead of avoiding inbreeding, have deliberately practiced it, making all

matings brother by sister within the litter. In this way advantage was taken of comparing two groups of animals, tests and controls, that were closely related and approaching homozygosity. In the pedigrees to be presented in a later paper it will be shown that all our animals are traced back to a single pair of rats. The advantages of this are obvious to the geneticist. It might be said that this experiment is now in the fifth generation of treated rats and in no way has inbreeding in our stock shown any ill-effects.

### Sterility Data

The daily treatment with alcoholic fumes for the period of a year beyond doubt affected the germ cells. Six alcoholic females were mated October 10, 1921, and in a period of six months only four litters had been born. Four of the six animals never produced a litter. Three of these at no time showed signs of pregnancy. The fourth, number 18, will be discussed later. Number 19 threw a litter on December 19, 1921, with five in the litter. One died on the first day and the rest died four days later. This animal did not breed again, although continuously mated until her death a year later. Female No. 20 produced all the young that carried on the alcoholic line. She threw two litters of normal size and weight, and a third litter of one. This animal was born with a large dark red spot on the back of its neck, evidently caused by a severe hemorrhage, and died on the third day. Forty-three days later alcoholic female No. 20 gave birth to a fourth litter of six normal-appearing young.

Five control females, sisters of the alcoholics, were mated at the same time. One of these proved to be sterile. The four productive controls in the same time produced 12 litters as compared to the five alcoholic litters produced by alcoholic female No. 20. That is, there were 86 control offspring to 26 alcoholics. The one fertile alcoholic, therefore, proved to be a slightly better breeder than the average of the controls.

Number 18, an alcoholic female, showed very different effects from the treatment. From the time of mating (October 10, 1921) to January 7, 1922, she was pregnant three times, and each time the litter was slowly absorbed. She became pregnant again, and on February 1, 1922, she aborted. This was followed by three more absorptions. In June, 1922, this female was taken from her alcoholic mate and mated with a normal male that was known to have sired several normal litters. The following month she gave birth to a still-born litter of one, which was normal in appearance except that it was smaller than the usual size of newborn rats. In fact, it is somewhat doubtful whether this was a late abortion or an undersized full term fetus. No. 18 was mated to normal males until her death November 28, 1922, but showed no further signs of pregnancy.

Of the six treated females of the first generation, therefore, three were rendered completely sterile; one had its germ cells so injured that the single litter it threw died in a few days; one had suffered less injury, but was not able to bring any of her eight litters, with the possible exception of one, to full term; while the sixth produced four litters, of which three were normal.

Both Pearl and Danforth ('19) get a higher percentage of sterility in the treated stock than in the controls. The treated rats appeared no different in life than did the controls; and when killed and examined showed no deposits of fat around the ovaries that would in any way inhibit ovulation. The sterility then must have been occasioned by the treatment with alcohol.

Although our animals were severely treated no abnormalities occurred such as Stockard observed, other than that of the hemorrhage noted above. One alcoholic litter, normal in appearance, died from some unknown cause within four days after birth.

It is commonly believed that after an injury to or alteration of the germ cells there is a tendency for them to revert to their normal condition after the lapse of a

greater or lesser period of time. And there is evidence that this is sometimes the case. However, when the injury is severe as in our experiment and the treatment extends over a considerable period of time it seems doubtful if recovery is always made. Five of the alcoholic females were mated for a period of something over nine months after the cessation of the treatment, and one, No. 18, was mated continuously with both alcoholic and normal mates for a year after the treatment ceased, with no evidence of germ cell recovery.

The data for growth in body weight, birth weight, litter size and sex ratio, for five successive generations of alcoholic and test animals are now ready for tabulation and will be published in subsequent papers of this series. The experiment will be continued through the tenth generation according to present plans, and a second series of reports made at the end of the tenth generation. Data secured for the first five generations indicates strongly the wisdom of long-continued experiments upon this important subject of induced changes in germinal materials.

LITERATURE CITED[8]

Bell, A. G.
1914. Sex determination in sheep. *Jour. Hered.*, 5, No. 2, p. 47.
Castle, W. E.
1906. The effects of inbreeding, cross-breeding, and selection upon the fertility and variability of Drosophila. Proc. Am. Arts and Sci., 41, p. 731.
Cole, L. J. and Davis, C. L.
1914. The effect of alcohol on the male germ cells, studied by means of double matings. *Science*, N. S., 39, pp. 476–477.
Danforth, C. H.
1919. Evidence that germ cells are subject to selection on the basis of their genetic potentialities. *Jour. Exp. Zool.*, 28, p. 385.
Donaldson, H. H.
1915. The Rat: Reference Tables and Data. Memoirs of the Wistar Institute of Anatomy and Biology, No. 6.
East and Jones.
1919. Inbreeding and Outbreeding. Philadelphia.
Hatai, S.

[8] The full bibliography will be published only in connection with this first report.

1915. The growth of organs in the Albino rat as affected by gonadectomy. *Jour. Exp. Zool.*, 18, p. 1.

Jackson, C. M.

1913. Postnatal growth and variability of the body and various organs of the Albino rat. *Am. Jour. Anat.*, 15.

Jennings, H. S.

1920. Life and Death, Heredity and Evolution in Unicellular Organisms. Boston.

King, H. D.

1918a. Effects of inbreeding on the growth and variability of body weight of the albino rat. *Jour. Exp. Zool.*, 26, p. 55.

1918b. Effects of inbreeding on the fertility and on the constitutional vigor of the albino rat. *Jour. Exp. Zool.*, 26, p. 55.

1922. Report of the Second International Congress.

Pearl, R.

1916a. On the effect of continuous administration of certain poisons to the domestic fowl, with special reference to progeny. Proc. Am. Phil. Soc., 55, p. 243.

1916b. Some effects of the continued administration of certain poisons to the domestic fowl, with special reference to progeny. Proc. Nat. Acad. Sci., 2, p. 675.

1917. The experimental modification of germ cells. Parts I, II and III. *Jour. Exp. Zool.*, 22, p. 125, p. 165 and p. 241.

Stockard, C. R.

1916. A further analysis of the hereditary transmission of the degeneracy and deformities by the descendants of alcoholized mammals. AM. NAT., 50, Part I, p. 50; Part II, p. 144.

1918. Further studies on the modifications of the germ cells in mammals: The effect of alcohol on treated guinea pigs and their descendants. *Jour. Exp. Zool.*, 26, p. 119.

Wright, Sewall.

1922. Report of the Second International Eugenics Congress.

# THE PSYCHODIDAE (MOTH-LIKE FLIES) AS SUBJECTS FOR STUDIES IN BREEDING AND HEREDITY

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## INTRODUCTION

A BATTERY jar containing decaying aquatic vegetation was noticed to contain a large number of midge-like larvae and pupae, and when minute moth-like flies belonging to two different species emerged from the pupa cases the jar was covered to see whether the flies would not breed. The possibility was suggested by the writer having inbred blow flies (*Calliphora vomitoria*) for a number of generations in an unsuccessful attempt to secure mutants. Within a few days it was noted that a new generation of larvae had been produced. These were isolated and a careful record kept of the specimens. Sixteen days after the first adults were seen a new generation of adults emerged from the isolated larvae. From these new adults fresh cultures were started, and after two generations of inbreeding a considerable number of white-eyed mutants were observed.[1] The second species of fly completed its life cycle in a somewhat longer time and produced no mutations. These results demonstrated the feasibility of using the animals for breeding experiments. The two species and the white-eyed mutation have been isolated and maintained as pure strains, the method of inheritance of the white-eyed mutation has been worked out and the reactions of normal and mutant flies recorded. It is intended that the present paper shall be one of a series giving an account of the results obtained.

[1] The writer is indebted to Dr. J. M. Aldrich, of the United States National Museum, for identifying the two insects described in this paper.

### REQUISITES OF SUITABLE BREEDING ANIMALS

Animals which have proved best adapted for studies in breeding and in life histories have been selected principally for the following reasons:

(1) They have been easily obtained. Rare animals and animals with obscure life histories have sometimes been reared with painstaking efforts, but the very difficulty of obtaining them and of maintaining them has precluded their general use.

(2) They have been easily reared and controlled. The preparation of peculiar media has sometimes presented such difficulties that the raising of the animals has been practically out of the question for general purposes and frequently the necessity of changing or renewing the medium has offered still further difficulties. The ideal animal has been one which could live upon some natural medium.

It has been desirable also that the animals in question be handled with facility. Microscopic animals require the use of a lens in locating them and the use of a capillary pipette or other apparatus in handling them. Larger animals are handled more easily.

(3) They have been relatively small. The consideration of the size of the animal raises the practical problems of housing, feeding and handling. Even such small animals as mice and rabbits when required in great numbers are very expensive to the operator in time, effort and money. Colony houses must be built and the animals must be fed and otherwise cared for. In handling specimens the size is also an important factor. The ideal animal in this respect is one large enough to be seen without a lens and yet small enough to be housed in considerable numbers within a limited space.

(4) They have been animals with a relatively short life cycle. Studies in heredity have been made upon animals whose life cycle extends over several years, but obviously such studies are time consuming and an extensive study involving many generations is a practical impossibility.

An animal which produces several generations a year or a generation every few weeks permits an extensive study within a reasonable period and is much more suitable for general use.

(5) They have had recognizable secondary sexual characters. In counting progeny and in mating pairs the sexes must be readily recognized, and some convenient distinguishing characteristic is a necessity for rapid work.

(6) They have had definite variations in form, color or structure, or have given rise to mutations.

In addition to the above points the ideal animal would require a minimum amount of attention beyond the mating of pairs and the counting of progeny.

In brief, the animals most used for experimental work in breeding are common forms of relatively small size, living upon some natural medium, easily reared and handled, having a short life cycle and either possessing conspicuous variations or capable of giving rise to mutations.

### Adaptability of Psychoda alternata and Psychoda minuta for Breeding Experiments

The details of the life histories of these two Psychodids will be discussed later in this paper, but the features which make them ideal animals for breeding experiments will be taken up here.

(1) They are common insects and are to be found during the entire summer season around sewage, manure piles or decaying aquatic vegetation.

(2) They are easily reared in natural media. Cow dung, horse dung, decaying vegetation and agar made with an old hay infusion as a solvent have been used as media with success. Many generations have been carried through in test tubes and flasks plugged with cotton to keep the animals from escaping. They are easily transferred from one tube to another by making use of their phototropic reactions or by shaking.

(3) They are about 2 mm in length—so small that a hand lens is required at first for distinguishing the sexes. This small size is a disadvantage, but with practise the secondary sexual characters can be made out with the naked eye. The small size is a distinct advantage, however, in the saving of housing space.

(4) *Psychoda alternata* has an extremely short life cycle. At a temperature of 15°–18° C. the life cycle is from 16 to 21 days in length. At 22°–25° C. the average life cycle is from 12 to 15 days in length and at a temperature of from 27°–30° C. the average life cycle extends from 7 to 12 days. Thus there is the possibility of obtaining from 25 to 30 generations in a year.

(5) A mutation which has white eyes and non-pigmented Malpighian tubules appeared in *Psychoda alternata* after the second generation of inbreeding with a normal wild stock. The normal wild fly has reddish-brown eyes and Malpighian tubules, and a reddish-brown ocellus in the larva. In the larval stage the ocellus of the mutant is not pigmented. The mutation was found to behave as a Mendelian recessive and a pure strain is now being maintained.

*The qualifications, in brief, which would recommend Psychoda alternata for breeding experiments are that it is a common insect which may be easily reared in test tubes or flasks in dung or in decaying vegetation. It is capable of producing a generation every 10 to 15 days, under ordinary temperatures. It requires no attention beyond the mating and counting of specimens. It has already given rise to a white-eyed mutation.*

There are some features in the life histories of these Psychodids that are not in their favor as ideal experimental animals. Adult life of sexually mature individuals is of very short duration, and the animals may die before suitable mates can be obtained. Like many other insects the males emerge a day or two in advance of the females and the males may die before the females appear. This can be remedied by forcing the life history

to be carrried out in a short space of time when the males and females emerge very nearly together. Specimens copulate soon after they emerge from the pupa case and it is difficult to procure a female which has not already copulated if males are present. This difficulty can be met, however, by isolating specimens in the pupa stage and allowing them to emerge each in a separate tube.

### Literature on Life History, Habits and General Structural Features of Psychodidae

The life histories of several species of the Psychodidae have been studied and published. Some of these studies have dealt with limited phases of the life history, while others have been rather complete.

The work of Haseman (1907, 1908) has been the most complete. In his "Monograph of North American Psychodidae" and his "Notes on the Psychodidae" he gives a systematic scheme for identification and descriptions of the various species. He also gives many notes on the life history of the members of the group, including the time that elapses during each stage and the habits during the various stages.

Fullaway (1907) and Welch (1912) give rather full accounts of the life history of three species.

Muttkowski (1915) in his "New Insect Life Histories" describes the larval, pupal and adult stages of one species (*Psychoda cinera*) together with an account of the movements, feeding habits and methods of respiration in the various stages.

Dell (1905) gives a complete description of the external anatomy of all stages of *Psychoda sexpunctata* (a species found in England), and an account of the internal anatomy in the larval and pupal stages. Interesting notes are included on the habits of the larvae and adults relating to their methods of locomotion, feeding and breathing.

Miall and Walker (1895) give descriptions of the external anatomy, life history and habits of another English species (*Pericomia canescens*).

## LIFE HISTORY AND EXTERNAL STRUCTURE OF PSYCHODA ALTERNATA AND PSYCHODA MINUTA

*Adult stage:* The adult insects are about 2 mm in length. The entire body surface and wings are densely covered with long hairs. The mouth parts are rudimentary except for a pair of four jointed maxillary palps. The antennae have thirteen segments, each segment having a bulbous swelling and a whorl of long hairs, while the terminal four segments are small and closely united. There are no ocelli but two compound eyes, dark reddish-brown in color, are present, each bearing about 75 facets. The wings are sharply pointed and are carried in a sloping position. They are fringed with long hairs and each vein forms an axis along which shorter hairs are placed. The abdomen contains nine segments and is slender in the male but swollen in the female. In the male the last segment is flattened, partially bifurcated and bears a long pair of forceps. A smaller pair of forceps borne on the eighth segment is enclosed by the larger pair on the terminal segment. The genital appendages in the female are very small.

*Psychoda alternata* has a light tan-colored body with wings lighter and faintly mottled with black and white. *Psychoda minuta* differs from *Psychoda alternata* in having a uniform dark color on the body and wings, longer hairs and in being more slender in the body, wings and antennae.

The adults of *Psychoda alternata* make rather sustained flights when opportunity offers, but are capable of running about actively and of jumping. The flights of *Psychoda minuta* are short and nervous, and the insect seems to rely more upon running and jumping as a means of locomotion.

Normal specimens of *Psychoda alternata* are positively phototropic for a period after emerging from the pupa case but seem to lose their sensitivity to light after copulation. They are also negatively geotropic. The latter reaction is strong in both normal and in white-eyed mu-

tants, but is of short duration, being confined to the first few hours of emergence. The males emerge in advance of the females and live only for a period of a few days. Females have been observed to live as long as seven days, but this is only in case they have not copulated and have retained their eggs. Normally they do not live so long.

During the reproductive activities the males come into contact with either males or females and after a little preliminary "testing" with the tips of the antennae they extend the forceps forward beneath the abdomen at the same time flexing the abdomen. They attempt to seize the fly with which they have come into contact and if successful will then turn about in the opposite direction but will still cling with the forceps. Males will seize other males quite as readily as they will seize females and one case was observed where males were actively running about seizing each other in a culture in which no females had yet emerged. This would tend to prove that sex recognition does not exist. They will not, however, even try to seize other objects, although males of one species will seize either males or females of another species. The adults are apparently sexually mature at the time of emergence. Copulation was observed in one instance between individuals only forty minutes after they had emerged from their pupa cases.

Dell (1905), after making the observation that the mouth parts are atrophied, states that the flies do not feed in the adult stages (*Psychoda sexpunctata*). Muttkowski in describing the habits of *Psychoda cinera* states that the adults feed continually. The writer has cleared a great many specimens of *Psychoda alternata* in absolute alcohol and xylol and has seldom failed to find a fluid in the alimentary tract corresponding in color to the medium upon which the animals were reared, indicating that there is some active feeding.

*Egg stage:* The eggs are laid in an oval-shaped, gelatinous mass from .5 to 1 mm in length. The eggs are

colorless and are arranged irregularly in the matrix. Almost any kind of decaying vegetation, sewage or dung furnishes the necessary chemical stimulus to induce the females to lay their eggs. Under ordinary conditions of temperature and moisture the eggs hatch in a little less than two days.

*Larval stage:* It is in this stage that the animal lives the longest. The young larva is at first entirely colorless but soon develops brown ocelli and a reddish brown pigment in the Malpighian tubules. Still later chitinous plates develop on the dorsal side which are quite brown at first but as the larva grows older they become darker. In addition to the head, which has a heavy chitinous covering, there are eleven body segments, each equipped with short setae. The effective mouth parts consist of a pair of two-jointed, bristled mandibles. The other mouth parts are distinct but rudimentary. The antennae are present as a pair of small knobs. The terminal body segment bears the anus on its ventral side and mounted on a long conical prominence a pair of tracheal openings which are encircled by a whorl of long hairs. Another minute pair of tracheal tubes may be found near the anterior end of the larva, but they do not open to the outside and are apparently functionless.

The alimentary canal is well developed, comprising an esophagus with a pair of salivary glands, a stomach and an intestine. There are no intestinal caeca, but five Malpighian tubules are attached to the intestine. These tubules are quite long in the larva and extend from the anterior to the posterior end of the body cavity being folded at least once.

The life habits of the larvae differ in the various members of the group, but all are alike in being active feeders. Some live under stones in swiftly running, well aerated water and are to be regarded as strictly aquatic. Others are semi-aquatic, living in the decaying aquatic vegetation floating on the surface of lakes and ponds, while other species are still further removed from the

aquatic habit, living in decayed tubers and roots. The two species considered in this paper are of the semi-aquatic type. They are capable of living in a pure fluid for a time, but seem to require a solid or semi-solid substratum upon which to move. The posterior end is raised so that the posterior spiracles may obtain air. It is very difficult to drown the larvae as the spiracles may, even if pushed below the water, entrap a bubble of air in the hairy circle about the opening and carry this down with them. Specimens have been submerged for nearly two days without harm to them, but they will eventually drown. Most of the locomotion is accomplished by creeping, but if a specimen is placed in shallow water it will sometimes move by grasping objects at the bottom with its mandibles and pulling itself along while the posterior end continues to be at the surface. The larvae are voracious feeders and devour whatever comes to hand and which they are capable of taking. Small bits of decaying vegetation, living and dead filaments of algae, moulds, sediment and a miscellaneous mass of smaller plants and animals are consumed. The length of the larval life varies greatly with conditions of moisture and temperature. Low temperatures and drying both tend to prolong the life cycle. Haseman found that *Psychoda cinerea* went through a life cycle in from three to five weeks during the late spring and summer while Muttkowski states five to six weeks to be the length of the life cycle in the same species earlier in the season. *Psychoda alternata* completes its life cycle in 8 to 24 days at temperatures ranging from 15° to 30° C. In specimens completing their cycles in 16 days, 10 or 11 days were passed in the larval stage. The maximum size attained by the larva is about 9 mm. No accurate account of the number of moults that take place in the larval stage has been kept, but larval casts measuring six and eight millimeters have been found.

*Pupal stage:* Towards the close of its larval life the insect ceases to feed, becomes sluggish and undergoes

the structural changes which convert it into a pupa. The most noticeable external features that arise at this time are the shortening of the entire body and the great differentiation in the head and thoracic regions. The entire chitinous covering becomes darkened, but especially so in the region of the head and the thorax. In *Psychoda alternata* this color may become a dense black while in *Psychoda minuta* it is brownish. The compound eyes arise early in the pupal life and the ocelli disappear. During the pupal life external markings appear which outline the positions of the developing palpi, antennae, legs and wings. The respiratory system becomes radically changed. In the transformation of the abdomen the terminal abdominal spiracles and trachea are lost and a new pair of organs is developed for securing air. A pair of hollow club-shaped organs arises at the anterior end of the body into which the trachea project. There are some other minor changes in the respiratory system. The alimentary canal shortens and differentiates and the Malpighian tubules also shorten.

The abdominal portion of the pupa is capable of movement, but there is little locomotion. The head and thorax are quite rigid and incapable of moving. During the two or three days of the pupal life the animal lies with its respiratory organs projecting from the water or sand and if by any chance these respiratory chambers are completely submerged the pupa will die.

*Period of emergence:* At the conclusion of the pupal stage the pupa case splits along a line between the head and thorax and the adult emerges. The operation of emergence takes about two minutes. The newly emerged specimen has control of its body, with the exception of its wings, and it immediately runs to a dry unobstructed place where the wings unfold. The wings start to straighten out at the base and the process proceeds to the tips, leaving the wings in a vertical position over the animal's dorsal side and parallel to each other. The straightening operation takes about ten seconds.

Within three minutes the wing-hairs become dry and the wings are dropped to their sloping position. The animal then proceeds about its main business of life, namely, reproduction, and has sometimes accomplished this within an hour after emergence.

### Culture Methods

*Media:* A number of different substances have been tried as media. The specimens were first noticed to breed in decaying aquatic vegetation composed principally of Potomogetan. It was recognized that a most successful medium would have to contain whatever chemical substance would induce the female to deposit her eggs and at the same time furnish food for the larvae. A variety of vegetable materials in a decaying condition were tried and all with some degree of success. Maple and oak leaves soaked in water and allowed to decay gave good results. Fleshy leaves—lettuce, spinach, cabbage—after becoming thoroughly broken down by bacteria and yeasts were successfully employed. Tubers and roots occasionally gave good results.

Some investigators have found different species of Psychodidae emerging from old horse and cow dung. These materials were tried as media and have proved so advantageous for the two species described in this paper that other media have been practically abandoned. Both old and fresh dung have been tried, and old dung has given the best results. The material is easily obtained and after being heated in order to kill the eggs or larvae of other insects or of other Psychodidae it may be placed in vessels and supplied with sufficient water.

Another preparation was made by filtering infusions of rotting vegetation or dung and dissolving in this fluid a quantity of agar sufficient to render it viscous. Bits of filter paper were also added to give the adults and larvae a "footing." The females will deposit their eggs readily in this preparation, and when the objective is to observe the animal during the various stages of its life history the medium has the advantage over the natural dung as

it is more transparent. Not many individuals come to maturity, however, in this medium, and it is useless where an entire brood must be brought to the adult stage.

*Moisture and temperature:* It is important to have the material upon which the larvae are to feed very moist and yet not covered with water. It will be recalled that the larvae have spiracles at the posterior end of the body which they keep at the surface of the water. Should the material upon which they feed be too deeply flooded the larvae can not keep the spiracles at the surface and at the same time feed. In most flooded preparations the larvae have been drowned after a few days. Ideal conditions are brought about by having the water at such a level that the base of the food mass is flooded and a portion of it projects above the surface. If the culture becomes too dry such individuals as come to maturity are small and weak and a full brood is never produced.

It has already been stated that conditions of temperature affect the length of the life cycle. A number of cultures have been maintained at various temperatures to determine the optimum temperature, one which will cause a life cycle to be passed through in a short time and yet one which would not injure the specimens. 25° C. has proved to be the optimum temperature. At low temperatures the adults are very sluggish and often do not copulate. It seems that the rôle of sight has little to do with the mating of the adults and that they depend upon actually coming into contact. Any agency, therefore, which can concentrate them into a small space and make them more active will promote copulation and insure a fertile culture. At low temperatures the larvae are slow to develop and the time of the life cycle is lengthened. At temperatures higher than 25° C. the adults are active and copulation takes place readily. However, the animals are affected adversely by the heat so that small broods are produced and various fluctuating variations are produced in the adults, such as dark mottlings on the body, short or abnormal wings, hairlessness, etc.

*Culture vessels:* Battery jars have proved to be very satisfactory for housing large mass-cultures. Beyond the renewing of the food material and water at intervals they require but little attention.

For experiments in which an accurate account of each individual must be kept such large vessels as battery jars can not be used. Cultures may be handled in large test tubes or small flasks filled about one third full of moist food material and plugged with cotton. Adults may then be transferred to other tubes and a record kept of the sex and character as each adult emerges. The tubes and flasks have the added advantage of confining the mating specimens to a small space so that contact is assured and copulation is induced. This is especially important in the case of white-eyed mutants. The normal adults are negatively geotropic and have a strong positive phototropic reaction. As a result of these two reactions the normal adults swarm together at the top of the flask on the light side. White-eyed mutants do not have the phototropic reaction and react to gravity poorly. They are reasonably active but have a tendency to remain scattered and in a large vessel they may miss contact altogether.

*Methods of isolating and transplanting:* The adults copulate within a very short time after they emerge from the pupal case and it hence becomes very important to isolate them as soon as they emerge. This is a laborious task and an almost impossible one, for great numbers will sometimes emerge within a very short time. This is of no consequence if the newly emerged adults are not to be mated. If, however, they are to be used for mating they must be isolated before the females have copulated. It has been the practise to isolate specimens desired for mating in the pupal stage, placing the pupa singly upon a wet cotton wad in the bottom of a test tube and plugging the tube with cotton. This insures that the adults when they emerge will not have been exposed.

In transplanting individuals from one culture to another or in obtaining adults from a mass culture it is

convenient to place over the culture jar a glass funnel and over the end of the funnel to place a test tube. If a strong light is placed above the tube the flies will react both to gravity and to the light and will pass through the neck of the funnel into the test tube in which they may be transferred.

## LITERATURE LIST

Banks, Nathan.
1907. Proc. Ent. Soc. Wash., No. 8, p. 148.

Banks, Nathan.
1914. Two new species of Psychoda. *Entom. News,* 25, 127–128.

Comstock, J. H.
A Manual for the Study of Insects, 428–29.

Dell, John Alexander.
1905. The structure and life history of Psychoda sexpunctata. Trans. Entom. Soc. of London for 1905, 293–311.

Fullaway, David T.
1907. Immature stages of a Psychodid fly. *Entom. News,* 18, 274–88.

Haseman, Leonard.
1907. A Monograph of North American Psychodidae, including ten new species and an aquatic Psychodid from Florida. Trans. Amer. Entom. Soc., Vol. XXXIII, 299–333.
1908. Notes on Psychodidae. *Entom. News,* Vol. XIX, 274–285.

Miall, L. C. and N. Walker.
1895. Life history of Pericomia canescens (Psychodidae). Trans. Ent. Soc. London, pp. 141–153.

Müller, Fritz.
1895. Notes on the immature stages of Brazilian Psychodidae. Trans. Ent. Soc. London.

Muttkowski, R. A.
1915. New insect life histories (Psychoda cinera Banks). Bull. Wis. Nat. Hist. Soc., Vol. 13, No. 2, pp. 109–116.

Welch, Paul S.
1912. Observations on the life history of a new species of Psychoda. Ann. Ent. Soc. America, 5, pp. 411–18.

Williston, Samuel W.
1908. Manual of North American Diptera. Psychodidae, pp. 92–93.

## SHORTER ARTICLES AND DISCUSSION

### THE RELATION OF MENDELISM TO MUTATION AND EVOLUTION

It has been generally assumed that Mendel's law of heredity affords a complete explanation of the facts of inheritance. But the characters which conform with Mendel's law, as Mendel understood it, involving dominance and segregation in 3:1 ratios, are comparatively few. They also relate to the more superficial, less important and most recently evolved characters of organisms. Very frequently they consist in the loss of some characteristic of the normal individual and so may be regarded as monstrous or defect variations. The English geneticists have been inclined to regard all Mendelian characters as due to loss variations, but this extreme position is scarcely tenable. Nevertheless, very few Mendelizing variations have a survival value equal or superior to that of the forms from which they have sprung. It is, therefore, probably the fate of most Mendelizing mutations to perish under natural conditions without contributing to racial evolution. But under domestication they are frequently preserved and incorporated in domestic races, if they happen to please man's fancy, which they sometimes do, just because they are distinctive.

The Mendelizing characters of domestic mammals consist very largely of color mutations, such as albinism (an all-white condition), or spotting, or a change from a complex gray and protectively colored coat to a simple black, brown or yellow coat. Other Mendelian mutations involve a change in the length of the hair or the hair slope. The hair may even practically disappear, as in the Mexican hairless dog, due to a Mendelizing dominant character.

Aside from their utility as breed trade-marks, color variations have little value in animal husbandry. The really valuable characters are those affecting size, proportions, rapidity of growth, milk yield, butter-fat content of the milk, wool production in sheep and fecundity, especially in swine and poultry. So far as differences exist between breeds in these important characters, they are not typically Mendelian in inheritance but blending. There is neither dominance nor segregation in recognizable Men-

delian ratios when such differences exist between the races crossed. Are they, then, Mendelian? For, if they are not, Mendel's law can not be regarded as the fundamental law of heredity. Very likely they are Mendelian. That is the current interpretation which I see no reason to reject until a better explanation can be substituted for it. (1) It is supposed that in the inheritance of blending characters genetic units (genes) are concerned which are not subject to the law of dominance. Such absence of dominance is known to occur in cases which are otherwise simple and Mendelian in character. (2) It is further supposed that blending characters depend upon the action of more than one gene, usually of numerous genes located in different chromosome pairs, so that they are independent of each other in transmission. The result is that complete segregation is a rare event. (3) It is supposed also that some of the genes on which blending characters depend are more important than others in determining the varying grades of expression of the character. Besides major, there are also minor or modifying factors. Such modifying factors are known to be operative in the inheritance of characters simply Mendelian, but varying slightly in expression.

The important outstanding question concerning evolution is: How do new variations arise? The most popular present-day answer to the question is *by mutation.* This answer, when first given by DeVries, was thought to involve sudden large changes made simultaneously in many characters of the organism giving rise from the start to distinct specific types. This sort of mutation does indeed occur in at least several groups of plants and the mechanism of it is now understood, as a result of the investigations of Gates and others on the evening primroses, of Harrison and others on the roses, and of Blakeslee on the jimson-weed. Such mutation involves variation in the germ cell through duplication of whole chromosomes or through the loss of whole chromosomes, involving as they do simultaneous changes in all characters which have genetic representation in the particular chromosomes duplicated or lost. This kind of mutation can not be regarded as a satisfactory general explanation of the origin of species, the thing which DeVries had in mind when he proposed the theory because comparatively few species differ from each other by whole chromosomes, or are incapable of interbreeding even if they do.

The current theory of mutation, which we owe largely to Morgan, is a theory of evolution by mutation in single genes, not in

whole chromosomes. It assumes that changes in single genes occur spontaneously without any as yet known external causes; that change in one gene does not necessarily involve change in any other gene and is usually not attended by it. Each gene mutation will obviously have to stand the test of natural selection and will consequently either be eliminated or will be added to the genetic complex of a surviving race. Intensive study of any species of animal usually reveals the occurrence within it of gene mutations with appreciable frequency. Students of Drosophila have calculated a rate for it in that species. A state of genetic flux may therefore be assumed to be the normal condition in an organism, which assumption corresponds exactly with the view of Darwin that heritable variation is ever present as material for natural selection to act upon. The contrary assumption made in the popular pure line doctrine is quite unsupported by recent critical experimental investigations as well as by the whole science of paleontology.

If each gene, as is apparently the case, is subject to independent mutation, it follows that a blending character, which by hypothesis depends on the joint action of many independent genes, will vary only gradually, since mutation in a gene at a time will produce only minor changes. Mutation in such cases will not be mutation at all except in name, but will consist of a gradual change in the direction favored by selection, again exactly the view of Darwin concerning the usual course of evolution.

The net result of our inventory is to show that Darwin was substantially right in his views concerning the nature of variation and the results of selection, and that the mutationists have lost their case except in so far as they have succeeded in attaching a new name to an old idea.

But we have acquired in Mendel's law a fundamental explanatory principle applicable to all heredity. Yet we must not expect simple three to one ratios to be found in all cases of inheritance or even in any considerable part of them. For only newly evolved characters will conform with it, such as are as yet represented only in a single gene in a single chromosome pair. Older, better established characters, those which characterize the species (rather than a variety), the genus or the class, will have become rooted in many or all chromosomes, and consequently in crosses with other species, genera or classes (when possible) will give a blended result.

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34

## CHROMOSOME ARRANGEMENTS DURING MEIOSIS IN CERTAIN OENOTHERAS

DURING the past two years I have been studying the pollen mother cells of pedigreed strains of the following Oenothera species: *Oe. franciscana sulfurea,*[1] *Oe. biennis, Oe. biennis sulfurea, Oe. muricata* and *Oe. oblonga.* The following is a brief account of certain meiotic stages in these plants. Fuller descriptions I hope to present in the near future.

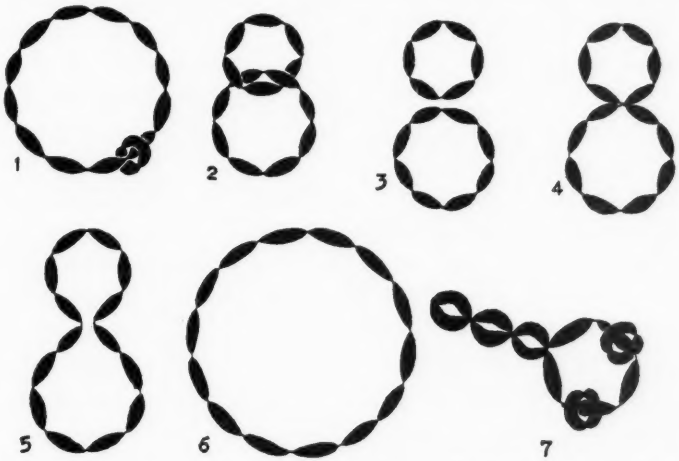
I have recently described the reduction process in the anthers of *Oe. franciscana.*[2] Late heterotypic prophase stages in this plant (corresponding to "diakinesis" in most plants) reveal an interesting situation, characterized by (1) a marked tendency on the part of the homologous chromosomes to become paired, a condition quite in contrast to that in most of the Oenotheras so far studied; (2) a constant and definite arrangement of the chromosomes in the nucleus; (3) the association of certain of the chromosomes end to end throughout the period, in such a way as to form a closed circle.

In regard to the first of these characteristics, we find that the species in question differ markedly from one another. On the one hand, *Oe. oblonga* shows a large amount of pairing of homologous chromosomes. *Oe. franciscana sulfurea,* on the other hand, succeeds in forming but one pair; and in *biennis, biennis sulfurea* and *muricata,* there is no pairing whatever. With respect to the other two characteristics, however, the plants show a striking uniformity. All present at this stage an arrangement of chromosomes in the nucleus which is constant for the species; and all display at least one large closed circle, consisting of a definite number of chromosomes united end to end. The arrangement of the chromosomes at this stage may be most briefly portrayed in a series of diagrams (see Figs. 1–7).

In the case of *muricata,* the arrangement shown in the figure persists unchanged until metaphase. In *franciscana sulfurea,* the single ring usually breaks away from the large circle, just previous to metaphase; otherwise, the arrangement persists. In *biennis* and *biennis sulfurea,* the typical arrangement (Fig. 2) may become somewhat modified by a separation of the two rings, or by an attachment of the two in such a way as to form a figure

[1] A fuller account of this species is soon to appear in the *Botanical Gazette.*

[2] *Amer. Jour. Bot.,* 9: 391–413. 1923.



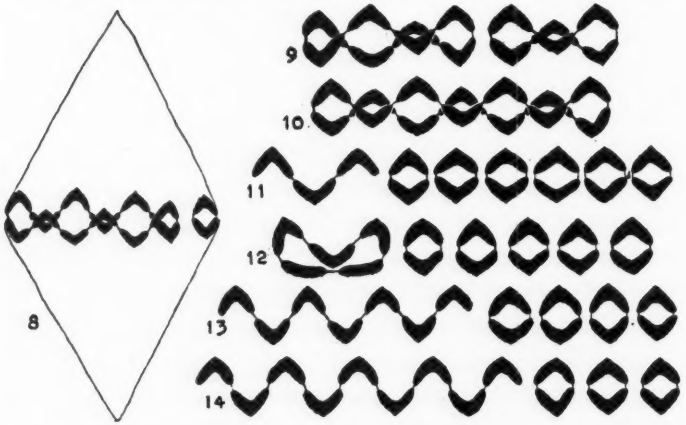

FIGS. 1–8. Diagrams of chromosome arrangements in late heterotypic prophase. 1, *Oenothera franciscana sulfurea;* 2, *Oe. biennis,* typical; 3, 4, 5, modifications of fig. 2: 6, *Oe. muricata;* 7, *Oe. oblonga,* probable complete arrangement.

8, or rarely, by an opening of the latter figure at the point of attachment of the two circles, so that the whole becomes one large circle of 14.

The situation in *oblonga,* a 15 chromosome form, is not so clear, owing to the fact that the chromosomes begin to break apart soon after the second contraction period has past. The complete grouping being rarely seen, therefore, the evidence for a definite arrangement of the chromosomes is not so complete as in the case of the other species. However, I think that the evidence is clear enough to make it fairly certain that the arrangement herewith presented is the correct one (Fig. 7). When the chromosomes separate, and the figure breaks up, the end result is not always the same. It appears that three sets of homologous chromosomes are always formed into definite pairs. In the case of the other chromosomes, however, the affinity between homologues appears to be weaker, and they sometimes succeed in pairing, and sometimes do not. The largest number of distinct pairs that I have observed in a nucleus is six. Such a large number of separate pairs can only be formed by the breaking up of the circle of 5 chromosomes, two of the components pairing. In most cases,

however, the five chromosomes which form the circle remain attached throughout this period, and the number of pairs formed is less than six. When the number of pairs formed is less than five, the chromosomes which failed to pair are found still attached to the circle of five, which breaks open, so that chains of seven or nine chromosomes are frequently seen (see Figs. 11–14). In all these species, therefore, there seems to be a definite arrangement of chromosomes in late heterotypic prophase, the arrangements differing in different species, but being constant in each. There is also in each species at least one closed circle, the number of chromosomes composing it varying from four in *franciscana,* to the whole group of fourteen in *muricata.*

Turning now to the heterotypic metaphase, we find that the chromosomes are arranged in the equatorial region as illustrated in the figures (Figs. 8–14). Chromosomes that were attached together in circles or chains in late prophase, are still attached, and those that were separated into pairs are still separated. But the most noticeable feature of this stage is the regular way in which the chromosomes forming the large circles are arranged. With but a small percentage of irregularity, contiguous chromosomes become attached to spindle fibres leading to opposite poles, alternate ones to fibers leading to the same pole. Thus it is brought about that in spite of the fact that the chromosomes fail




FIGS. 8–14. Diagrams of chromosome arrangements in heterotypic metaphase, lateral view. Outline of the spindle omitted except in fig. 8; 8, *Oe. franciscana sulfurea;* 9, *Oe. biennis;* 10, *Oe. muricata;* 11–14, *Oe. oblonga.*

in large measure to pair, but instead, remain attached to one another end to end, they are for the most part distributed evenly and normally to the poles.

The chromosomes in all the Oenotheras that I have studied are so similar in size and appearance that it is impossible to distinguish one from another, and so, by direct observation, determine the exact position of each chromosome pair. However, the uniform linking of groups of chromosomes in each species, and the regular way in which they are arranged in metaphase, and distributed in anaphase, suggests very strongly that the chromosomes making up the circles are not arranged by chance, but have positions in these circles which are definitely fixed. If this were not the case, there would be very little chance that the two members of each of the homologous pairs in the circles would be disjoined in anaphase, by the process employed in these plants. It is more than likely, therefore, that the chromosomes are placed in such a way in the circles, that homologous ones are separated to opposite poles. Bearing in mind the condition found in metaphase, the most natural arrangement allowing of such a result is an end to end relation between the homologous chromosomes, and this I believe is in all likelihood the actual condition. If we assume the probability that within the larger groups of chromosomes there is a constant arrangement of the homologous chromosomes into pairs, it is but an easy step to the further assumption that the chromosomes are not only arranged into pairs, but have definite positions within the pairs also. Thus the arrangement AA′ BB′ CC′ (representing three adjacent pairs of homologous chromosomes) should not, except through irregularity, become AA′ B′B CC′.

Supposing, then, that the chromosomes are thus fixed in their position in the circle, it follows clearly that the group as a whole constitutes a unit, both structurally and in behavior, acting very much like a single chromosome pair. Chromosomes ABCD normally go to one pole, and A′B′C′D′ to the other. Chromosomes ABCD are then as much linked in effect as though they were merely parts of a single chromosome, the homologue of which is composed of A′B′C′D′.

The possible bearing of this situation upon the experimental work of Shull[3] in which he finds evidence of a large amount of

[3] *Eugenics, Genetics and the Family,* Vol. I, pp. 86–99, 1923; *Genetics* 8: 154–167, 1923.

character linkage in the Oenotheras, need only be mentioned. Shull finds that most of the characters that he has studied so far seem to belong to the same linkage group. He believes, therefore, that the genes for all of these characters are carried in the same chromosome pair. In view of the facts here presented, it seems to me that the possible correspondence of larger units composed of several chromosomes, rather than of individual chromosome pairs, to character linkage groups such as Shull has discovered, should be kept in mind.

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## THE INGESTION OF CHLOROPHYL BY ANIMAL CELLS

WHILE the experiments on tissue cultures carried on in this laboratory for the past few years have shown that the connective-tissue cells of the chick embryo are able to take in a wide range of foreign bodies, nevertheless it is rather startling to be able to cause animal cells to become as green as any plant cell simply by placing them in an environment containing chlorophyl.

The size of the chlorophyl droplets formed in the animal cell depended upon the method of preparing the chlorophyl and varied from almost ultramicroscopic yellow green particles to large blue green droplets about the same size as the fat droplets native to the cells.

Chlorophyl obtained by boiling crushed plant (grass or spinach) tissue was toxic and this method was soon discarded, even though when a trace of this was added to the medium of the tissue cultures very minute green globules later appeared in the cells. Plant juice undergoes rapid and marked changes upon exposure to the air and becomes toxic, not only for animal tissue but also for plant cells. This has proved one of the great hindrances to obtaining plant tissue-cultures, for while almost any animal tissue exhibits some growth, however slight, in plasma cultures, plant tissue is rapidly killed when placed in plant juice.

If the plant juice was crushed in 95 per cent. alcohol a deep blue green transparent solution resulted which was not at all poisonous to animal cells, provided the alcohol was diluted or driven off by heat. When the alcoholic extract was added to the tissue culture medium (Locke-Lewis solution, Lewis and Lewis, 1915),[1] it formed a slightly turbid solution containing chloro-

[1] Lewis, M. R., 1918, *Science*, XLVIII, 398.

phyl in very minute globules. The cells of a 24 hour growth of embryonic chick connective tissue, bathed in such a solution containing not more than 1 per cent. alcohol, became (after 20 hours) a pale yellow green color due to the inclusion of a great many very small green globules. A much stronger emulsion of chlorophyl was obtained by adding 25–50 per cent. of the alcoholic extract to the medium and then holding a flame over it until the alcohol was burned off. This procedure left a deep blue green scum over the solution which broke up into globules upon cooling. This scum was drawn off and stirred in fresh medium until an emulsion of green globules about the size of yolk globules was formed. This was placed on cultures of connective tissue and was rapidly taken in by the cells, so that after twenty hours the cells contained many deep blue green globules of different sizes scattered through the cytoplasm.

As has been shown in regard to other foreign bodies the clasmatocytes exhibited the greatest avidity in the ingestion, often becoming so full of green globules that the other structures of the cell were obscured.

The behavior of the embryonic chick connective tissue in vitro toward these green globules was the same as they exhibited toward other foreign bodies, especially fat globules (Smith, 1921;[2] Lewis, 1918).[3] There were no purposeful movements exhibited by the cell toward the chlorophyl globules or vice versa. The contact between the foreign body and the cell took place entirely by chance. The cells made no effort to engulf the globules and no cell processes flowed out around them. In a few instances a vacuole was formed around one or a group of the chlorophyl globules. Little digestion of the chlorophyl took place, although occasionally some of the globules become yellow green and faded out, as though digestion had occurred. No green color appeared in any of the mitochondria nor were the chlorophyl globules ever attached to the mitochondria.

Cultures containing cells which had ingested chlorophyl lived as long as the controls, in some sets of experiments even longer, but no particular benefit to the cells was noted; they lived, divided and degenerated about as usual.

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[2] Lewis, M. R. and W. H. Lewis, 1915, *Amer. J. Anat.*, XVII, 339.

[3] Smith, D. T., 1921, Johns Hopkins Hosp. Bull., XXXII, 240.

## CYTOLOGICAL STUDIES IN THE GENERA RUBUS AND CRATAEGUS

CYTOLOGICAL investigations of known hybrids by European and American investigators have shown that hybrids can be separated from more stable species by their chromosome behavior at meiosis. Some authors even have been able to use this character to distinguish $F_1$ hybrids from hybrids of later generations.

The present cytological studies were undertaken in order to determine if some irregularity in the chromosome distribution of the pollen-mother-cell, during its development, would not explain the reproductive sterility so general in Rubus and Crataegus.

Based on studies of the pollen-mother-cell development in 36 species and forms of Rubus, and 81 of Crataegus, I sum up the situation as follows:

The Rubus can be divided into two major classes:

(1) Diploid species, in which I find the gametophytic chromosome number to be 7, the sporophytic 14.

(2) Polyploid species, including triploid, tetraploid, pentaploid, hexaploid and octoploid.

The diploid Rubi are characterized by a regular distribution of chromosomes and regular pollen formation. This, I consider, is a pretty reliable criterion of a homozygous species.

The polyploidous species are characterized, not only by an increase of chromosomes above the diploid number, but by striking irregularities in chromosome distribution and irregular pollen formation frequently leading to polycary and polyspory. In view of the similarity of these irregularities to those found in known hybrids, I consider that this group is made up of heterozygous species and forms.

The Crataegi can be divided into three major classes:

(1) Diploid species, in which I find the gametophytic chromosome number to be 16.

(2) Triploid and tetraploid species that are able to develop their pollen-mother-cells to the tetrad stage.

(3) Triploid and tetraploid species in which the pollen-mother-cell is thin and vacuolated and seldom develops to the tetrad stage.

The diploid Crataegi are characterized by a normal pollen formation.

The triploid and tetraploid species that are able to form pollen show irregularities in their chromosome distribution that frequently leads to polycary or polyspory. These characters are clearly of the type associated with heterozygous species.

The triploid and tetraploid species that are unable to form pollen represent hybrids that are unable to reproduce themselves and have resulted from crosses of distantly related species.

The diploid species of Rubus and Crataegus can be compared advantageously with the stable diploid species of Rosa as recently studied by Täckholm, and the stable *Oenothera grandiflora* and *O. franciscana* of the Onagraceae.

I have found polyploidous Rubi and Crataegi very general. This cytological study, substantiated by morphological and genetical studies, has shown the hybrid nature of a large number of species in these two polymorphic genera.

These heterozygous forms show the following characters: pollen sterility, variability of offspring, polyploidy, irregular chromosome distribution, polycary and polyspory—features all clearly associated with hybrid forms.

It seems, therefore, reasonable to conclude that in these two polymorphic genera, multiplication of species has taken place by hybridization in their natural habitats. Some hybrids may date back to preglacial times, but there is much evidence to show that many of the so-called species originate at the present time wherever inter-fertile species are present.

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## A GYNANDROMORPHIC BEE OF THE GENUS OSMIA

IN going over a collection of Osmias from the Rocky Mountain Region belonging to the American Museum of Natural History, an interesting gynandromorphic specimen was found. Although such phenomena have been frequently mentioned and discussed[1]

[1] See Morgan, T. H., "The Physical Basis of Heredity," pp. 189–93; also Cockerell, T. D. A., "Zoology," pp. 85–6. The only reference to a gynandromorphic Osmia which the writer was able to find occurs, page 91, "Die Hermaphroditen und Gynandromorphen Hymenopteren," by K. W. v. Dalla Torre and H. Friese.

This specimen was referred to *Osmia caerulescens* L. and is described as having the head mainly female, with the exception of the pubescence of the left side of the clypeus; thorax and abdomen male, but bluish as in the female; legs entirely male; abdomen with seven segments; genital organs apparently male.

as occurring in other genera, the occurrence is very unusual for this genus. In the key to the females of this region this specimen runs to *Osmia pentstemonis* Cockerell from which it differs only in having the legs dull black and not at all metallic as is true of *pentstemonis*; so the writer has assigned it to this species.

The following characters were noted: About 8–8.5 mm long; bilateral division apparent in the head and abdomen, but no apparent division in the thorax; the male characters occur on the right side and the female on the left. Right side of head brassy green with white hairs; antenna with thirteen joints, dark ferruginous beneath; mandible bidentate; cheek with black hairs above and some white hairs below. The vertex does not show such distinct division, but is bluish with black and white hairs intermixed. Left side of head blue with black hairs, which are almost true bristles; more coarsely punctured than the right side; antenna with twelve joints; mandible tridentate; hair of cheek entirely black. Thorax dark greenish blue; mesothorax and scutellum with punctures hardly the diameter of a puncture apart, shining between punctures; pubescence largely white with some black hairs intermixed; hair of pleurae apparently light, but badly worn; tegulae black. The sculpturing of the abdomen is similar on the two sides, but otherwise the sexual characters are clearly defined on each side. Right side of abdomen brassy green with pure white pubescence; apical segment somewhat emarginate at the side as in *eutrichosa* Cockerell, with a tuft of longer hairs at the side. Left side of abdomen blue with black pubescence; ventral scopa black. Wings faintly dusky; anterior wing 6.25 mm long; basal nervure slightly in front of the nervulus; marginal cell slightly fuscous; left wing with the second recurrent nervure reaching the second submarginal cell nearer the apex than in the right wing. Legs black, with pubescence largely dark.

Habitat: Peaceful Valley, Colorado, June 18, 1922, on dandelion (*Taraxacum*), (Dorothy Young). Collection American Museum of Natural History.

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## THE BEE-EATING PROCLIVITY OF THE SKUNK[1]

IN the August number of *Gleanings in Bee Culture*[2] for 1923,

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 229.

[2] Published by the A. I. Root Company, Medina, Ohio.

Mr. Geo. S. Demuth, one of the editors, (p. 546) has the following to say concerning the relation of the skunk (*Mephitis*) to *Apis mellifica:* "Skunks are sometimes quite destructive in the apiary. They scratch at the entrance to entice the bees to come out, and then eat them in large numbers. Apparently these animals have no fear of stings or have some method of eating the bees without being stung." In this connection the following observations may be of sufficient interest to be worth recording.

During the summer of 1923, while working on the biology of bumblebees, I had under observation about sixty *Bremus* (*Bombus*) colonies, of which all but one were kept in situations where they could not be molested by ants, the single exception being a colony of *Bremus fervidus* which had been transferred to the Bussey Beeyard and placed beneath a table on which a colony of hivebees was kept. On the morning of August 21, I discovered that the nest-box of this bumblebee colony had been completely cleaned out—apparently by some mammal—with the exception of a few shreds of cotton and about a half a dozen workers which were eagerly searching for their brood. For the benefit of these five or six workers, another *fervidus* colony, consisting of a queen and about thirty workers, was transferred to the spot. However, three days later (August 24), I found that this colony also had been completely destroyed during the preceding night. I therefore decided to ascertain the identity of the marauder, and in the evening set a trap baited with bumblebee comb. To my great satisfaction, I found a half-grown skunk in the trap on the following morning. Being interested to know how this animal is able to dispose of a whole bumblebee colony, apparently without being stung, I decided to keep it to see whether I could not make some observations on its bee-eating habits.

For this purpose the skunk was provided with a special domicile composed of two adjoining boxes, each measuring about 2 x 2 x 2 feet, one of which may be called compartment No. 1, and the other, compartment No. 2. Compartment No. 1, which was partly filled with hay, was completely closed, with the exception of a small opening (about 6 x 9 inches) which communicated with compartment No. 2, while compartment No. 2 was provided with a layer of sand and sawdust, and with a wide-mesh wire cover. In this way, I was able to place food in compartment No. 2 without running much risk of being sprayed, for

the animal habitually spent the day in compartment No. 1 and prowled about in compartment No. 2 at night, and it was also possible to make the following observations on its bee-eating habit.

On August 27, a colony of *Bremus fervidus,* consisting of a queen, about fifty workers, and some brood, was placed in compartment No. 2 shortly after dark (about 8 P. M., daylight-saving time). I waited until about 9 P. M., but the animal refused to leave its den (compartment No. 1). However, when I returned about a half an hour later and turned on my flashlight, the skunk was greedily eating a piece of comb containing brood in compartment No. 2. He did not seem to like this intrusion, however, for he immediately stopped eating and returned to his den. Under these circumstances it seemed best to discontinue my observations for that night. When compartment No. 2 was examined the next morning, nothing was left of the *fervidus* colony and nest, excepting a few shreds of cotton.

I now became interested to know whether a skunk can also overcome large bumblebee colonies. A nest of *Bremus impatiens,* containing at least 200 workers and numerous queens and males, was therefore placed in compartment No. 2 on August 28, shortly after dark. To my astonishment, this colony also was found completely destroyed on the following morning. This remarkable feat aroused my curiosity still more, and I decided to make a second attempt to see whether I could not find out how the skunk accomplishes such a hazardous task, for, according to my experience, half a dozen bumblebee stings are sufficient to put the average person to bed for a day or two.

In order to keep the animal from returning to its den after entering compartment No. 2, the sliding door between the two compartments was attached to a pulley so that the opening by which the two compartments were joined could be closed without much noise. After making this change, a colony of *Bremus impatiens,* consisting of about 75 workers, a few queens and males, and some brood, was placed in compartment No. 2 on August 30, shortly after dark. At about 8 P. M., the skunk entered compartment No. 2, whereupon the door between the two compartments was closed. When I turned on one of my two flashlights, the animal again tried to slink back into its den, and, finding the door closed, worked for over an hour at the latter trying to get back into compartment No. 1. However, about 9:15 P. M. the

skunk suddenly turned his attention to the bumblebee colony, and now the following, at times exceedingly comical, "melodrama"—the "music" was supplied by the bees—was enacted within two feet of my flashlights:

The first thing the skunk did was to scratch at the nest. This was immediately answered by a shrill chorus from within. As the workers came rushing out, they were seized one by one with one of the front paws, rolled on the ground for a few moments with a quick, alternating, back-and-forward movement of both paws, and then eaten with a crunching noise. At other times, the skunk would seize a piece of cotton with which the bees had covered their comb, roll it in the manner described, and then examine it to see whether it contained any bees. However, every once in a while he would make a frantic jump as a result of being stung by some worker which had eluded him. Such bees were usually seized directly with the teeth and eaten without being rolled. From time to time, the skunk tried to get at the comb, but in this he did not succeed until he had disposed of about half of the bees, when he quickly seized some of the brood, and after having carried it into a corner, began to devour it with much relish. While thus engaged, he was several times painfully interrupted by prowling workers. After he had consumed this chunk of brood, he returned to the nest, and after having disposed of several more bees, took out another piece of comb and ate it. By 10 P. M., all of the bees and comb had been disposed of in this manner, excepting one or two workers and several males which had made their escape by crawling through the wire cover of compartment No. 2. On one or two occasions, I saw him eat a male without rolling, but he invariably rolled the queens. When one of the latter accidentally fell into his water dish, he fished her out with one of his front paws and then treated her in the same manner as the others.

In the evening of September 1, this experiment was repeated before several students and a member of the faculty of the Bussey Institution with a somewhat larger colony of *Bremus impatiens*. Although the skunk failed to work as well on this occasion as two nights before, he had disposed of about a fourth of the colony and brood by 10 P. M., and by the next morning nothing remained of this colony and nest, except some cotton, a few empty cocoons and six or seven numb workers. On this occasion, the skunk was stung more frequently than on the pre-

ceding, but apparently without any ill after-effects, for on the following day (September 2) he readily ate beetles, crickets, grasshoppers, moths and even full-grown woolly bear caterpillars (*e.g., Arctia acraea;* det. Dr. A. L. Reagh). Of these insects, the woolly bears were rolled in a similar fashion as the bumblebees, but longer and more leisurely. The beetles, crickets, grasshoppers and moths, on the other hand, were consumed without this treatment. The non-insect part of a skunk's bill of fare seems to be well known,[3] and I shall therefore not discuss my rather scanty observations along this line.

In addition to the bumblebee colonies which were destroyed in the Bussey Beeyard, I found a considerable number of despoiled *Bremus* and *Vespa* nests in and near the Arnold Arboretum, all of which but one [4] bore distinct earmarks of having been destroyed by skunks.

## SUMMARY

(1) The skunk is an important enemy, not only of the hive-bee, but also of bumblebees and probably also of social wasps.

(2) The skunk fears stings, but the latter do not seem to have very serious after-effects.

(3) When robbing a bumblebee colony, the skunk makes most of the bees harmless by rolling them on the ground and then eating them.

O. E. PLATH

BOSTON UNIVERSITY

[3] Cf. Detlefsen, J. A. and Holbrook, F. M. "Skunk breeding with notes on mutations and their genetic behavior." *Journal of Heredity,* Vol. 12 (1921), pp. 243–254, 8 figs.

[4] This nest was a surface nest of *Bremus fervidus* and was probably destroyed by a crow or some other bird.

# INDEX

NAMES OF CONTRIBUTORS ARE PRINTED IN SMALL CAPITALS


ALLEN, GLOVER M., Geographic Distribution of New England Mammals, 97
Amphibians, Geographical Distribution of, E. R. DUNN, 129
Anura, Origin and Distribution of, MAYNARD M. METCALF, 385

BARBOUR, T., Reptiles in the East and West Indies, 125
BELLING, JOHN, Microscopical Methods used in Examining Chromosomes in Iron-Acetocarmine, 92
BLAKESLEE, ALBERT, and M. E. FARNHAM, Trisomic Inheritance, 481
BLANCHARD, FRANK N., History of Four-toed Salamander, 262
Blood-sugar Values in Heredity, OSCAR RIDDLE and HANNAH ELIZABETH HONEYWELL, 412
BREITENBECHER, J. K., Red-spotted, Sex-limited Mutation in Bruchus, 59
BRIMHALL, DEAN R., Family Resemblances among American Men of Science, 74, 137, 326
BRUES, CHARLES T., Geographical Distribution of Onycophora, 210

CALKINS, GARY N., Maupas and the Infusoria, 350
CALVERT, PHILIP P., Geographical Distribution of Insects, 218
CASTLE, W. E., Mendelism, Mutation and Evolution, 559
CHICKERING, A. M., Longitudinal Division of Tentacles of Hydra Fusca, 89
CHIDESTER, F. E., Cyclopean Monsters, 496
Chlorophyl, MARGARET REED LEWIS, 566
Chromosome Arrangements in Oenothera, RALPH E. CLELAND, 562
CLARK, HUBERT LYMAN, Distribution and Derivation of some New England Echinoderms, 229
CLAUSEN, ROY E., Inheritance in Drosophila Hydei, 52
CLELAND, RALPH E., Chromosome Arrangements, 562
Cyclopean Monsters, F. E. CHIDESTER, 496

Drosophila Hydei,, Inheritance in, ROY E. CLAUSEN, 52; Willistoni, Parallel Characters in, CHARLES W. METZ and RUTH M. FERRY, 381
DUNN, E. R., Geographical Distribution of Amphibians, 129
DUNN, L. C., Lethal Gene in Fowls, 345

EIGENMANN, CARL H., Fishes of Pacific Slope of South America, 193; Yellow Fever and Fishes, 443

Family Resemblances among American Men of Science, DEAN R. BRIMHALL, 74, 137, 326
FARNHAM, M. E., and ALBERT BLAKESLEE, Trisomic Inheritance, 481
FERRY, RUTH M., and CHARLES W. METZ, Parallel Characters in Drosophila Willistoni, 381
Fiddler Crabs, Asymmetry in, 269; Abdomen in Immature, T. H. MORGAN, 274
Fishes, of Pacific Slope of South America, 193; Yellow Fever and, C. H. EIGENMANN, 443
Fruit Breeding, Experimental Accuracy in, WALTER SCOTT MALLOCH, 435

Genes for Extension of Black Pigment in Chicken, WILLIAM A. LIPPINCOTT, 284
Geographical Distribution, of Certain New England Mammals, GLOVER M. ALLEN, 97; Ecological Aspects of Bird Distribution in Tropical Africa, JAMES P. CHAPIN, 106; Reptiles in East and West Indies, T. BARBOUR, 125; of Amphibians, E. R. DUNN, 129; Fishes of Pacific Slope of South America, CARL H. EIGENMANN, 193; of Onycophora, CHARLES T. BRUES, 210; of Insects and Age and Area Hypothesis of Dr. J. C. Willis, PHILIP P. CALVERT, 218; and Derivation of some New England Echinoderms, HUBERT LYMAN CLARK, 229

GONZALEZ, B. M., RAYMOND PEARL and SYLVIA L. PARKER, Duration of Life, 153, 289

HANSON, FRANK BLAIR, and VIRGINIA HANDY, Effects of Alcohol Fumes on Albino Rat, 532
HOLLAND, W. J., Vertebral Formula in Sauropoda, 477
HONEYWELL, HANNAH ELIZABETH, and OSCAR RIDDLE, Blood-sugar Values in Heredity, 412

Insects, Geographical Distribution of, PHILIP P. CALVERT, 218; Subcostal Vein in Wings of, ALEX. D. MACGILLIVRAY, 371

KEMPTON, J. H., Inheritance of Mesocotyl Length in Hybrids of Brachytic Maize, 374

Lethal Gene in Fowls, L. C. DUNN, 345
LEWIS, FREDERIC T., Symmetry as Factor in Evolution of Plants and Animals, 5
LEWIS, MARGARET REED, Chlorophyl, 566
Life, Duration of, RAYMOND PEARL, SYLVIA L. PARKER and B. M. GONZALEZ, 153; B. M. GONZALEZ, 289
LIPPINCOTT, WILLIAM A., Genes for Extension of Black Pigment in Chicken, 284
LONGLEY, ALBERT E., Rubus and Crataegus, 568

MACOMBER, DANIEL, Fertility in Rats, 519
MACGILLIVRAY, ALEX. D., Subcostal Vein in the Wings of Insects, 371
MALLOCH, WALTER SCOTT, Experimental Accuracy in Fruit Breeding, 435
Maupas and the Infusoria, GARY N. CALKINS, 350
Mendelism, Mutation and Evolution, W. E. CASTLE, 559
METCALF, MAYNARD M., Origin and Distribution of the Anura, 385
METZ, CHARLES W., and RUTH M. FERRY, Parallel Characters in Drosophila Willistoni, 381
Mitochondria Problems, IVAN E. WALLIN, 255
MORGAN, T. H., Asymmetry in Fiddler Crabs, 269; Abdomen in Immature Fiddler Crabs, 274; Absence of Lutear Cells in Testis of Male Phalarope, 476
MULLER, H. J., Simple Frequency Formula, 66
Mutation, Red-spotted, Sex-limited, in Bruchus, J. K. BREITENBECHER, 59

NOBLE, G. K., Chelys and Phylogeny of Turtles, 377
NUTTALL, GEORGE H. F., Symbiosis in Animals and Plants, 449

Onycophora, Geographical Distribution of, CHARLES T. BRUES, 210

PEARL, RAYMOND, SYLVIA L. PARKER and B. M. GONZALEZ, Duration of Life, 153
PLATH, O. E., Bee-eating Proclivity of Skunk, 570
Psychodidae, C. L. TURNER, 545

Rats, Fertility in, DANIEL MACOMBER, 519; Alcohol Fumes on Albino, FRANK BLAIR HANSON and VIRGINIA HANDY, 532
RIDDLE, OSCAR, and HANNAH ELIZABETH HONEYWELL, Blood-sugar Values in Heredity, 412
Rubus and Crataegus, ALBERT E. LONGLEY, 568

Salamander, Life History of Four-toed, FRANK N. BLANCHARD, 262
SANDHOUSE, GRACE ADELBERT, Gynandromorphic Bee, 560
Sauropoda, Vertebral Formula in, W. J. HOLLAND, 477
Specific Characters, Origin and Inheritance of, F. B. SUMNER, 238
STURTEVANT, A. H., and FRANZ SCHRADER, Sex Determination, 379
Subcostal Vein in Wings of Insects, ALEX. D. MACGILLIVRAY, 371
SUMNER, F. B., Origin and Inheritance of Specific Characters, 238
Symbiosis in Animals and Plants, GEORGE H. F. NUTTALL, 449
Symmetry in Evolution of Plants and Animals, FREDERIC T. LEWIS, 5

Trisomic Inheritance, ALBERT BLAKESLEE and M. E. FARNHAM, 481
TURNER, C. L., Psychodidae, 545
Turtles, Chelys and Phylogeny of, G. K. NOBLE, 377

WALLIN, IVAN E., Mitochondria Problem, 255
WRIGHT, SEWALL, Color Factors of the Guinea Pig, 42

Yellow Fever and Fishes, C. H. EIGENMANN, 443

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Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

## CONTENTS

PAGE

I. Trisomic Inheritance in the Poinsettia Mutant of Datura. DR. ALBERT BLAKESLEE and M. E. FARNHAM ..... 481

II. The Origin of Cyclopean Monsters. DR. F. E. CHIDESTER ..... 496

III. The Threshold of Fertility in Rats and its Relation to Diet Efficiency. DR. DANIEL MACOMBER ..... 519

IV. The Effects of Alcohol Fumes on the Albino Rat. FRANK BLAIR HANSON and VIRGINIA HANDY ..... 532

V. The Psychodidae as Subjects for Studies in Breeding and Heredity. C. L. TURNER ..... 545

VI. Shorter Articles and Discussion: The Relation of Mendelism to Mutation and Evolution: PROFESSOR W. E. CASTLE. Chromosome Arrangements during Meiosis in Certain Oenotheras: DR. RALPH E. CLELAND. The Ingestion of Chlorophyl by Animal Cells: DR. MARGARET REED LEWIS. Cytological Studies in the Genera Rubus and Crataegus: DR. ALBERT E. LONGLEY. A Gynandromorphic Bee of the Genus Osmia: GRACE ADELBERT SANDHOUSE. The Bee-eating Proclivity of the Skunk: DR. O. E. PLATH ..... 559

VII. Index to Volume LVII ..... 575

---

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CONTENTS OF NOVEMBER–DECEMBER NUMBER

The Progression of Life in the Sea. Dr. E. J. Allen.
Family Resemblances among American Men of Science. Dr. Dean R. Brimhall.
Autophoric Transplantation, its Theory and Practice. Professor Hans Przibram.
Spontaneous Metamorphosis of the American Axoloti. Professor W. W. Swingle.
Shorter Articles and Discussion: More Eyeless Cladocera: Dr. Arthur M. Banta and L. A. Brown. Crossingover Involving Three Sex-linked Genes in Chickens: A. S. Serebrovsky. A Fourth Allelomorph in the Albino Series in Mice: Dr. H. W. Feldman.
Index to Volume LVI.

CONTENTS OF JANUARY–FEBRUARY NUMBER

A Note on Symmetry as a Factor in the Evolution of Plants and Animals: Dr. Frederic T. Lewis.
Two New Color Factors of the Guinea Pig: Dr. Sewall Wright.
Inheritance in Drosophila hydei: Dr. Roy E. Clausen.
A Red-spotted Sex-limited Mutation in Bruchus: Dr. J. K. Breitenbecher.
A Simple Formula giving the Number of Individuals Required for Obtaining one of a Given Frequency: Professor H. J. Muller.
Family Resemblances among American Men of Science: Dr. Dean R. Brimhall.
Shorter Articles and Discussion: Longitudinal Division of the Tentacles of Hydra fusca: Dr. A. M. Chickering. Microscopical Methods used in Examining Chromosomes in Iron-acetocarmine: Dr. John Belling.

CONTENTS OF MARCH–APRIL NUMBER

SYMPOSIUM ON GEOGRAPHICAL DISTRIBUTION
Geographic Distribution of Certain New England Mammals. Glover M. Allen.
Ecological Aspects of Bird Distribution in Tropical Africa. James P. Chapin.
Reptiles in the East and West Indies—and Some Digression. T. Barbour.
The Geographical Distribution of Amphibians. Dr. E. R. Dunn.
Family Resemblances among American Men of Science. Dr. Dean R. Brimhall.
Experimental Studies on the Duration of Life. Professor Raymond Pearl, Sylvia L. Parker and B. M. Gonzales.

CONTENTS OF MAY–JUNE NUMBER

SYMPOSIUM ON GEOGRAPHICAL DISTRIBUTION
The Fishes of the Pacific Slope of South America. Professor Carl H. Eigenmann
The Geographical Distribution of the Onycophora. Professor Charles T. Brues

CONTENTS OF MAY–JUNE NUMBER—*Continued*

The Geographical Distribution of Insects and the Age and Area Hypothesis of Dr. J. C. Willis. Dr. Philip P. Calvert
The Distribution and Derivation of Some New England Echinoderms. Dr. Hubert Lyman Clark
The Origin and Inheritance of Specific Characters. Dr. F. B. Sumner
The Mitochondria Problem. Ivan E. Wallin
The Life History of the Four-tied Salamander. Dr. Frank N. Blanchard
The Development of Asymmetry in the Fiddler Crab. Professor T. H. Morgan
Variation in the Width of the Abdomen in Immature Fiddler Crabs. Professor T. H. Morgan
Shorter Articles and Discussion: Genes for the Extension of Black Pigment in the Chicken: Professor William A. Lippincott

CONTENTS OF JULY–AUGUST NUMBER

Experimental Studies on the Duration of Life. Dr. Bienvenido Maria Gonzalez.
Family Resemblances among American Men of Science. Dr. Dean R. Brimhall.
A Lethal Gene in Fowls. Dr. L. C. Dunn.
What did Maupas mean? Professor Gary N. Calkins.
Shorter Articles and Discussion: The Subcostal Vein in the Wings of Insects: Professor Alex. D. MacGillivray. Inheritance of Mesocotyl Length in Hybrids of Brachytic Maize. Dr. J. H. Kempton. Chelys and the Phylogeny of the Turtles: G. K. Noble. A Note on the Theory of Sex Determination: Drs. Franz Schrader and A. H. Sturtevant. The Parallel Characters "Crossveinless" and "Vermilion" in Drosophila willistoni: Dr. C. W. Metz and Ruth M. Ferry.

CONTENTS OF SEPTEMBER–OCTOBER NUMBER

The Origin and the Distribution of the Anura. Professor Maynard M. Metcalf.
The Behavior of Blood-sugar Values in Heredity. Dr. Oscar Riddle and Dr. Hannah Elizabeth Honeywell.
Experimental Accuracy in Fruit Breeding. Walter Scott Malloch.
Yellow Fever and Fishes. Professor T. H. Eigenmann.
Symbiosis in Animals and Plants. Professor George F. Nuttall.
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